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CÂMPUS DE JABOTICABAL

ISOLAMENTO, CARACTERIZAÇÃO E IDENTIFICAÇÃO DE RIZOBACTÉRIAS COM HABILIDADES PARA PROMOVER O CRESCIMENTO DE PLANTAS

Roberta Mendes dos Santos

Tecnóloga em Produção Sucroalcooleira

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Orientador: Prof. Dr. Everlon Cid Rigobelo

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Microbiologia Agropecuária

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TÍTULO DA TESE: ISOLAMENTO, CARACTERIZAÇÃO E IDENTIFICAÇÃO DE RIZOBACTÉRIAS COM HABILIDADES PARA PROMOVER O CRESCIMENTO DE PLANTAS

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“Não há saber mais ou saber menos: Há saberes diferentes.”

Paulo Freire

À Deus, pela força, coragem e persistência, durante esta caminhada.

E aos meus pais!

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ISOLAMENTO, CARACTERIZAÇÃO E IDENTIFICAÇÃO DE RIZOBACTÉRIAS COM HABILIDADES PARA PROMOVER O CRESCIMENTO DE PLANTAS

RESUMO – As rizobactérias promotoras de crescimento de plantas têm o potencial para melhorar a produtividade das culturas. Os principais mecanismos pelos quais essas bactérias podem estimular o crescimento das plantas incluem, a produção de fitormônios, disponibilização de nutrientes e produção de compostos orgânicos voláteis e antimicrobianos, tais como antibióticos, enzimas líticas e sideróforos. Vários isolados bacterianos são relatados com o potencial de estimular o crescimento em diferentes culturas e sob diversos sistemas de produção agrícola e vêm sendo utilizados como inoculantes na agricultura, com a finalidade de melhorar a produtividade das culturas. Neste contexto, o presente estudo teve o objetivo isolar, caracterizar e identificar bactérias provenientes da rizosfera de cana-de-açúcar e analisar seu potencial de crescimento em plantas de cana-de-açúcar e milho. O isolamento das rizobactérias foi realizado a partir de solo rizosférico de seis diferentes variedades de cana-de-açúcar, coletado nas cidades de Jaboticabal – SP, Pirajuba – MG e Frutal – MG. A seleção das cepas bacterianas ocorreu a partir da realização de testes *in vitro* qualitativos e quantitativos de fixação biológica de nitrogênio, produção de ácido indolacético, atividade celulolítica e solubilização de fosfatos e potássio. Os isolados selecionados foram identificados a partir da extração e amplificação do rDNA 16S. Enquanto que, o potencial de promoção de crescimento e colonização de sete isolados foi avaliado a partir de testes em casa de vegetação com cana-de-açúcar e milho. Foi avaliado altura, massa seca, teores de fósforo e nitrogênio nas plantas e contagem de unidades formadoras de colônias nas plantas e no solo. Foram isoladas 167 cepas bacterianas que apresentaram habilidades de promoção de crescimento, destas, sete cepas foram selecionadas e identificadas pertencendo aos gêneros, *Staphylococcus*, *Enterobacter*, *Bacillus* e *Achromobacter*. Cinco isolados conseguiram proporcionar o crescimento em cana-de-açúcar, *Enterobacter* sp. IP11, *Enterobacter* sp. IP14, *Bacillus anthracis* IP17, *B. thuringiensis* IP21, *Achromobacter spanius* IP23. Na cultura do milho, somente *Enterobacter* sp. IP14, *B. anthracis* IP17 e *B. thuringiensis* IP21 promoveram o crescimento. A maioria das cepas bacterianas isoladas da rizosfera de cana-de-açúcar tiveram uma preferência pela mesma cultura. Entretanto, é possível e foi verificado com esse estudo, que rizobactérias isoladas de cana-de-açúcar podem também ser capazes de promover o crescimento e o desenvolvimento vegetal do milho.

Palavras-chave: inoculantes, promoção do crescimento de plantas, rizosfera, *Saccharum* spp., sustentabilidade, *Zea mays*

ISOLATION, CHARACTERIZATION, AND IDENTIFICATION OF RHIZOBACTERIA WITH ABILITIES TO PROMOTE PLANTS GROWTH

ABSTRACT - Plant growth-promoting rhizobacteria have the potential to improve crop productivity. The main mechanisms by which these bacteria can stimulate plant growth include the production of phytohormones, availability of nutrients, and production of volatile organic compounds and antimicrobials, such as antibiotics, lytic enzymes, and siderophores. Several bacterial isolates are reported with the potential to stimulate growth in different crops and under different agricultural production systems and have been used as inoculants in agriculture, with the purpose of improving crop productivity. In this context, the present study aimed to isolate, characterize, and identify bacteria from the sugarcane rhizosphere and to analyze their growth potential in sugarcane and maize plants. The rhizobacteria isolation was carried out from rhizospheric soil of six different sugarcane varieties, collected in the cities of Jaboticabal - SP, Pirajuba - MG, and Frutal - MG. The selection of bacterial strains occurred through the performance of qualitative and quantitative in vitro tests for biological nitrogen fixation, indoleacetic acid production, cellulolytic activity, and phosphate and potassium solubilization. The selected isolates were identified from the extraction and amplification of the 16S rDNA. The potential for promoting growth and colonization of seven isolates was evaluated using greenhouse tests with sugarcane and maize. Height, dry mass, phosphorus, and nitrogen levels in plants, and counting of colony-forming units in plants and soil were evaluated. 167 bacterial strains that showed growth promotion abilities were isolated, of these, seven strains were selected and identified belonging to the genera, *Staphylococcus*, *Enterobacter*, *Bacillus*, and *Achromobacter*. Five isolates were able to provide growth in sugarcane, *Enterobacter* sp. IP11, *Enterobacter* sp. IP14, *Bacillus anthracis* IP17, *B. thuringiensis* IP21, *Achromobacter spanius* IP23. In maize, only *Enterobacter* sp. IP14, *B. anthracis* IP17, and *B. thuringiensis* IP21 promoted growth. Most bacterial strains isolated from the sugarcane rhizosphere preferred the same culture. However, it is possible and it has been verified with this study, that rhizobacteria isolated from sugarcane may also be able to promote the growth and plant development of maize.

Keywords: inoculants, plant-growth promotion, rhizosphere, *Saccharum* spp., sustainability, *Zea mays*

CAPÍTULO 1 - Considerações gerais

1. Introdução

O aumento da produção agrícola para atender às demandas dos mercados consumidores e da crescente população mundial, depende do uso de uma grande quantidade de fertilizantes e pesticidas químicos, que muitas vezes são usados em excesso no solo (Kumar et al., 2017). O uso destes produtos na produção agrícola proporciona um aumento médio da produtividade de aproximadamente 50% em relação à produção sem o seu uso; no entanto, as práticas de fertilização química ignoram o potencial biológico das raízes e da rizosfera, aumentando a mobilização e aquisição de nutrientes, porém diminuindo as interações entre as plantas e os microrganismos rizosféricos (Meena et al., 2017). Muitos estudos demonstram as habilidades de microrganismos promotores de crescimento de plantas (MPCP) para aumentar o estado nutricional da planta e reduzir o uso de pesticidas e fertilizantes químicos (Aloo et al., 2019).

Dentre os MPCP, se destacam as rizobactérias promotoras de crescimento de plantas (RPCP), esse termo foi definido pela primeira vez por Kloepper e Schroth (1978), para descrever bactérias do solo que colonizam a rizosfera das plantas e que estimulam seu crescimento através de vários mecanismos. Seu uso é uma forma potencial de diminuir os impactos ambientais negativos, resultante do uso contínuo de pesticidas e fertilizantes químicos.

As RPCP são uma excelente alternativa para auxiliar os agricultores frente aos novos desafios da agricultura moderna, mantendo a alta produtividade das culturas e impactando o mínimo possível o meio ambiente (Pérez-Montaño et al., 2013). Essas rizobactérias promovem o crescimento e aumento da produtividade das plantas utilizando mecanismos diretos e indiretos (Figura 1).

Os mecanismos diretos estão ligado à produção de fitormônios e à disponibilização de nutrientes, como fósforo (P), nitrogênio (N) e ferro (Fe), através da solubilização de fosfatos, fixação biológica de nitrogênio (FBN) e da produção de

sideróforos, respectivamente (Riggs et al., 2001; Cassán et al., 2009; Krey et al., 2013; Yu et al., 2019). Os mecanismos indiretos estão relacionados ao biocontrole, por meio da atividade antagônica contra microrganismos fitopatogênicos, induzindo assim, respostas de resistência sistêmica da planta, interferindo nos sistemas de *quorum sensing* (QS) bacteriano. Alguns relatórios mostram que as RPCP podem usar mais de um desses mecanismos para realizar o aprimoramento do crescimento das plantas (Bashan e Holguin, 1997; Ahmad et al., 2016).

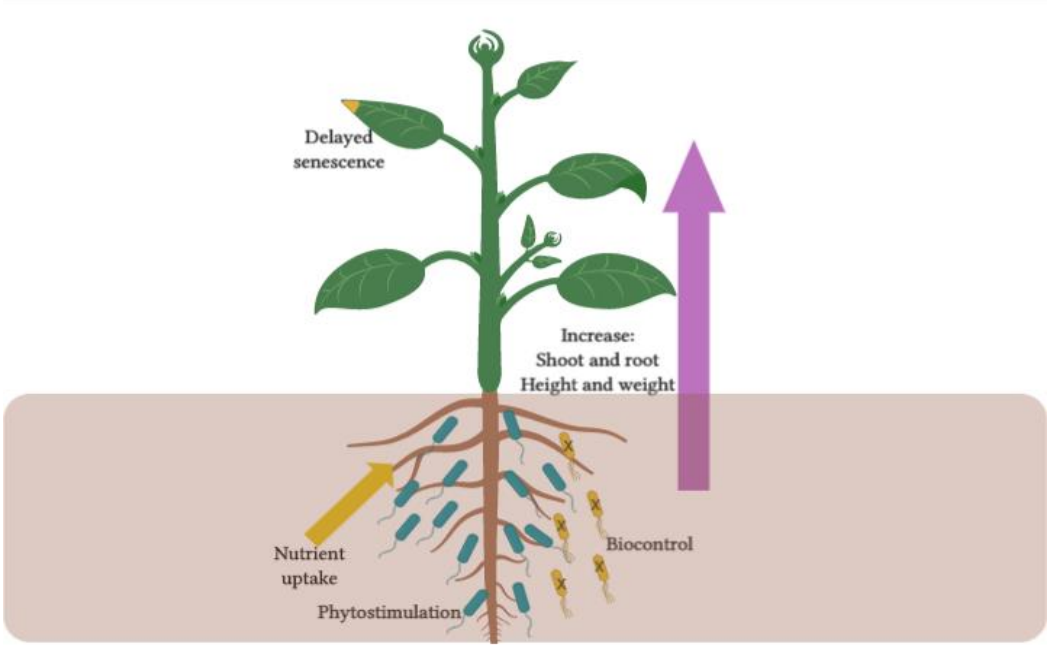

Figura 1. Benefícios decorrentes de interações entre RPCP e plantas hospedeiras. Esses benefícios incluem, aumento na taxa de germinação, na massa e comprimento de parte aérea e raízes, aumento de área foliar, conteúdo de clorofila, absorção de nutrientes, conteúdo de proteína, além de melhora na produtividade, tolerância a estresses bióticos e abióticos, biocontrole e retardado da senescência das plantas (Santos et al., 2020).

A colonização competitiva da rizosfera é crucial para que, as bactérias benéficas possam exercer seus muitos mecanismos de ação. Este processo de colonização e estimulação do crescimento pelas RPCP pode estar sujeito a mecanismos estritos de reconhecimento e sinalização molecular entre a bactéria e a planta hospedeira (Kamilova et al., 2006). Esses mecanismos de reconhecimento são mediados por exsudatos vegetais específicos que atraem as bactérias para a rizosfera do hospedeiro sendo que, esses exsudatos podem servir como fonte de carbono e

atuar como moléculas sinalizadoras (Albareda et al., 2006). Assim, as RPCP devem ter a capacidade de adesão às sementes ou raízes das plantas para posterior colonização e um processo competitivo que é afetado pelas características genotípicas das RPCP e da variedade da planta hospedeira (Muñoz-Rojas e Caballero-Mellado, 2003).

Além disso, a determinação específica da composição da comunidade bacteriana da rizosfera através da identificação dessas bactérias, é importante, propiciando a identificação dos grupos e o conhecimento de seus metabolismos, possilitando ainda o descobrimento de associações de microrganismos importantes para o desenvolvimento e crescimento vegetal (Pedrinho et al., 2010).

Neste contexto, o presente estudo teve o objetivo de isolar, caracterizar e identificar bactérias oriundas da rizosfera de cana-de-açúcar e analisar seu potencial para promover o crescimento de plantas de cana-de-açúcar e milho.

2. Revisão de literatura

2.1 Rizosfera

A rizosfera é definida como a zona ao redor das raízes das plantas que é diretamente influenciada por suas secreções (Hartmann et al., 2009). A planta, o solo e os microrganismos interagem entre si para realizar e influenciar vários processos que contribuem para a saúde e produtividade da planta (Ahmed e Holmström, 2015).

Para que as RPCP colonizem a rizosfera, são necessários processos de seleção, pelos quais a microbiota bacteriana das raízes é diferenciada do bioma do solo circundante. Um dos processos se refere às atividades de desenvolvimento e secreção de moléculas a partir do sistema radicular vegetal, sendo denominado de rizodeposição. Este constitui um mecanismo molecular potencial à formação de uma microbiota distinta da rizosfera e dos biomas do solo.

As células da rizoderme secretam uma ampla gama de compostos, incluindo íons de ácidos orgânicos, íons inorgânicos, fitosideróforos, açúcares, vitaminas, aminoácidos, purinas e nucleosídeos, e a capa da raiz produz um polissacarídeo, conhecido como mucilagem (Dakora e Phillips, 2002). A rizodeposição parece alimentar uma mudança inicial da comunidade microbiana orientada pelo substrato na rizosfera, que converge com o ajuste fino dependente do genótipo do hospedeiro e dos perfis da microbiota na seleção microrganismos próximos as raízes (Bulgarelli et al., 2013).

Sugere-se que esses processos modulam, em vários graus durante o desenvolvimento da planta, o estabelecimento de comunidades microbianas na rizosfera (Dini-Andreote e Raaijmakers, 2018). A regulação da microbiota rizosférica é impulsionada não apenas pelas plantas, mas também pelos próprios microrganismos, podendo modular o ambiente da planta e até mesmo reprogramar a planta para sua vantagem por meio da exsudação de fitohormônios, compostos orgânicos voláteis, moléculas com *quórum sensing* (QS) e antimicrobianos (Venturi e Keel, 2016).

2.2 Mecanismos utilizados pelas RPCP

2.2.1 Mecanismos diretos

A ação direta dos microrganismos promotores do crescimento de plantas envolve a melhoria do solo, através de benfeitorias na sua fertilidade, com o aumento de nutrientes essências, como nitrogênio, fósforo, potássio e ferro (Naik et al., 2019). Essas melhorias incluem ainda, a produção de substâncias para o crescimento das plantas, tais como fitormônios reguladores de crescimento vegetal (Tabassum et al., 2017) (Figura 2).

2.2.1.1 Produção de fitormônios

Fitormônios são pequenas moléculas orgânicas que desempenham um importante papel no desenvolvimento do crescimento das plantas e permitem que elas tolerem diferentes condições de estresse (Shaterian et al., 2005). Algumas rizobactérias podem produzir diferentes tipos de fitormônios, incluindo auxinas, citocininas, giberelinas, etileno e ácido abscísico (ABA), que agem em diferentes processos de crescimento das plantas, incluindo a multiplicação celular, que resulta em aumento da extensão radicular (Glick, 2014; Kaur et al., 2016). No entanto, a produção de ABA por rizobactérias é também considerada uma forma indireta de promoção de crescimento (Belimov et al., 2014).

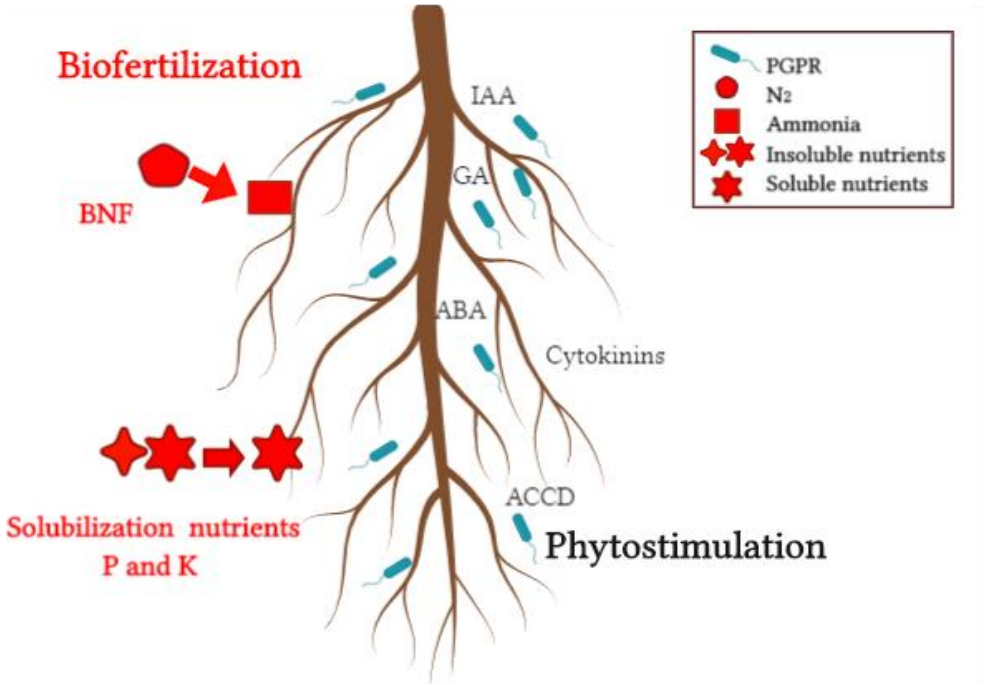

Figura 2. Mecanismos diretos que beneficiam o crescimento da planta a partir de interações das RPCP com as plantas hospedeiras. Biofertilização, através da solubilização de nutrientes e FBN, e fitoestimulação, a partir da produção de auxinas, citocininas, giberelinas, etileno e ABA (Santos et al., 2020).

2.2.1.1.1 Auxinas

As auxinas influenciam muitos aspectos do crescimento e desenvolvimento das plantas (Halliday et al., 2009; Grossmann, 2010). A auxina natural mais crucial (e a mais conhecida) é o ácido indolacético (AIA), produzido por bactérias, plantas e fungos. Nas plantas, esse hormônio desempenha um papel essencial na divisão celular, alongamento, desenvolvimento de frutos e senescência. Este estimula o desenvolvimento das raízes, folhas e flores (Phillips et al., 2011) e, particularmente, nas dicotiledôneas, induz especificamente a formação das raízes laterais, enquanto nas monocotiledôneas induz a formação das raízes adventícias (McSteen, 2010).

O desenvolvimento das plantas é afetado pelo AIA de maneiras favoráveis e prejudiciais, e muitas bactérias têm a capacidade de sintetiza-lo, incluindo bactérias benéficas e fitopatógenos (Duca at al., 2014). Supõe-se que mais de 80% das bactérias isoladas da rizosfera sejam capazes de sintetiza-lo (Patten e Glick, 1996; Khalid et al., 2004), sendo sua produção parte do sistema de sinalização e comunicação entre plantas e bactérias presentes na rizosfera (Spaepen et al., 2007).

O principal precursor para a síntese desse hormônio é o triptofano e a adição deste aminoácido aos meios de cultura, resulta em todos os casos em sua maior produção. A biossíntese do triptofano começa a partir do corisma dos tecidos meristemáticos, em uma reação de cinco etapas codificada pelos genes *trp* (Merino et al., 2008).

As vias de síntese do AIA dependentes de triptofano incluem as vias indol-3-acetamida, indol-3-piruvato, triptamina e indol-3-acetonitrila (Spaepen et al., 2007), e embora alguns intermediários possam diferir, a maioria das vias mostra similaridade com aquelas descritas em plantas (Patten e Glick, 1996; Woodward e Bartel, 2005). As suas vias de síntese foram identificadas usando vários métodos genéticos e bioquímicos, no entanto, apenas um pequeno conjunto de genes e enzimas envolvidos nessas vias foi caracterizado (Spaepen e Vanderleyden, 2011).

A correlação entre a concentração desta auxina e o crescimento das plantas não é linear, sendo que, as plantas têm níveis ótimos de AIA endógenos para o seu melhor desenvolvimento (Duca et al., 2014). Quantidades excessivas podem provocar

efeitos deletérios nas plantas, como a inibição do crescimento radicular (Duca et al., 2014). Portanto, o uso deste hormônio para estimulação do crescimento de plantas deve ser cuidadosamente regulado para evitar efeitos inibitórios de super dosagem. Nesse contexto, as plantas possuem mecanismos de neutralização para controlar o excesso de AIA, como sua inativação por conjugação com açúcares, aminoácidos ou peptídeos (Sitbon et al., 1992). No entanto, nem sempre as plantas conseguem realizar essa neutralização e por vezes são prejudicadas pelo seu excedente.

Aeromonas punctata (Iqbal e Hasnain, 2013), *Azospirillum brasilense* (Camilios-Neto et al., 2014), *Bacillus subtilis* (Tahir et al., 2017), *Burkholderia phytofirmans* (Poupin et al., 2016), são algumas espécies bacterianas eficientes na síntese de AIA.

2.2.1.1.2 Citocininas

As citocininas são pertencentes ao grupo de reguladores de crescimento e responsáveis pela formação da parte aérea, inibição do alongamento radicular, melhoria na divisão celular e desenvolvimento radicular (Porcel et al., 2014, Jha e Saraf, 2015). Em particular, elas são obrigatórias para a progressão do ciclo celular (Schaller et al., 2014), além disso, o equilíbrio entre auxinas e citocininas determina o funcionamento do meristema, a arquitetura do sistema radicular, a formação de órgãos laterais da parte aérea e o desenvolvimento de órgãos reprodutores (Schaller et al., 2015). As citocininas regulam ainda, a biossíntese da clorofila e a biogênese dos cloroplastos (Cortleven e Schmulling, 2015), e estão envolvidas no desenvolvimento da resistência das plantas a estresses bióticos e abióticos (Grosskinsky et al., 2011; O'Brien e Benkova, 2013).

Vários tipos de citocinas são produzidos por rizobactérias, nas quais a zeatina e a cinetina são as mais abundantes. As rizobactérias sintetizam a zeatina por via direta e indireta. A via direta envolve a síntese de dimetilalil difosfato e isopenteniladenosina monofosfato, enquanto a via indireta envolve *cis*-zeatina contendo tRNA para liberar citocininas (Tabassum et al., 2017).

Algumas cepas de *Azotobacter* spp., *Rhizobium* spp., *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, *B. subtilis* e *Paenibacillus polymyxa* são capazes de produzir citocininas (de Salamone et al., 2001; Glick, 2014).

2.2.1.1.3 Giberelinas

Outra classe essencial de fitormônios liberados pelas rizobactérias são as giberelinas, responsáveis por diferentes processos de desenvolvimento, como germinação de sementes, processo de floração, alongamento do caule e frutificação em plantas superiores (Saleem et al., 2015). As giberelinas regulam positivamente a divisão e o alongamento celular, estimulando o crescimento do hipocótilo e do caule, e têm um efeito positivo no tamanho da folha e no tamanho do meristema radicular (Martinez et al., 2016). A giberelina exógena pode estimular o crescimento da parte aérea, o desenvolvimento do xilema e inibir o crescimento radicular (Guo et al., 2015, Wang et al., 2015).

Estudos têm mostrado que plantas com a presença de bactérias produtoras de giberelinas em suas rizosferas têm melhores taxas de crescimento (Poupin et al., 2013; Vacheron et al., 2013). Algumas espécies bacterianas produtoras de giberelinas incluem, *Bacillus amyloliquefaciens* (Shahzad et al., 2016), *Enterococcus faecium* (Lee et al., 2017), *Sphingomonas* sp. (Khan et al., 2014), *Bacillus pumilus* (Joo et al., 2004).

2.2.1.1.4 Etileno

O etileno provou ser, em baixa concentração, potencialmente ativo para maturação de frutos e folhas, germinação de sementes, senescência foliar, murcha de flores, iniciação, alongamento e ramificação das raízes, formação de nódulos e abscisão de folhas. Enquanto que, em concentrações mais altas, o etileno pode ser

tóxico para as plantas, causando desfolhamento, inibição do crescimento das raízes e senescência em estágio prematuro (Desbrosses et al., 2009). Quando a planta passa por algum estresse, como frio, infecção, inundação, seca e até a presença de metais potencialmente tóxicos, ela passa a produzir o precursor do etileno, ou seja, 1-aminociclopropano-1-carboxilato (ACC) (Reid, 1988; Li et al., 2005; Liu et al., 2013).

A ACC-deaminase (ACCD) é uma enzima produzida por algumas bactérias, sendo esta enzima benéfica para as plantas devido sua ação na redução dos níveis de ACC, clivando-o em amônia e α-cetobutirato. Essa quebra ajuda a regular os efeitos adversos do excesso de etileno, reduzindo seus níveis. Portanto, as rizobactérias capazes de produzir ACCD são benéficas para plantas cultivadas em condições de estresse, como a seca (Sandhya et al., 2010), alta salinidade (Mayak et al., 2004), metais potencialmente tóxicos (Belimov et al., 2001), regulando os níveis de ACC da planta, reduzindo o etileno a níveis não tóxicos.

Azotobacter spp., (Dubey et al., 2012; Farajzadeh et al., 2012), *Bacillus* spp., (Belimov et al., 2001) e *Pseudomonas* spp. (Sandhya et al., 2010; Kamran et al., 2016), são algumas espécies conhecidas pela produção de ACCD.

2.2.1.1.5 Ácido abscísico

O ácido abscísico (ABA) é um fitormônio envolvido na mediação do fechamento estomático (Mansfield et al., 1990), na regulação de aspectos do crescimento e desenvolvimento das plantas na ausência de estresse (Cheng et al., 2002). O ABA é produzido por plantas, algas, bactérias e fungos (Zeevaart, 1999).

O estresse hídrico estimula elevados níveis de biossíntese do ABA pelas plantas e isso causa o fechamento parcial dos estômatos, como uma resposta adaptativa para conservar a água (Dodd, 2007). O ABA pode afetar ainda, a inibição da germinação das sementes, indução da senescência das plantas e a abscisão de folhas e frutos (Munemasa et al., 2015, Sah et al., 2016).

O ABA é também conhecido por reduzir o crescimento das plantas, embora seja necessária uma certa quantidade de ABA para o crescimento, uma vez que este

hormônio regula a abertura estomática e, por conseguinte, a perda de água e absorção de CO_2 (Pospisilova, 2003). Algumas RPCP podem reduzir os níveis de ABA na planta hospedeira e, indiretamente, aumentar o crescimento da planta, sendo que esses efeitos positivos dependem dos níveis endógenos de ABA da planta hospedeira (Belimov et al., 2014).

Várias rizobactérias produzem ABA em meios de cultura ou mediam o *status* de ABA das plantas (Dodd et al., 2010), sendo *Achromobacter xylosoxidans* (Forchetti et al., 2007; Sgroy et al., 2009), *A. brasilense* (Cohen et al., 2008), *Bacillus licheniformis*, *B. subtilis, Brevibacterium halotolerans e Pseudomonas putida* (Sgroy et al., 2009), algumas destas rizobactérias.

2.2.1.2 Fixação biológica de nitrogênio

O nitrogênio (N) é o nutriente mais limitante para o desenvolvimento das plantas, e pode ser assimilado do solo na forma de nitrito, nitrato ou amônia. Essas formas de N não são abundantes na maioria dos solos e a fertilização química com N empregada na agricultura é frequentemente perdida durante a chuva ou pela lixiviação dos fertilizantes minerais nitrogenados (Pérez-Montaño et al., 2014).

Nesta circunstância, as bactérias desempenham um papel fundamental, devido ao fato que, algumas podem realizar a fixação biológica de nitrogênio (FBN). Esses microrganismos fixadores de N são classificados em dois grupos diferentes, microrganismos simbióticos e os de vida livre (Gopalakrishnan et al., 2017). A FBN é realizada por um conjunto de genes específicos conhecidos como genes *nif*, que junto com outros genes estruturais participam na ativação da proteína de Fe, doação de elétrons, biossíntese do cofator de Fe molibdênio e muitos outros genes reguladores obrigatórios para a síntese e atividade enzimática (Reed et al., 2011).

A FBN pode ocorrer através da formação de nódulos com formação de simbiose ou com bactérias de vida livre. As bactérias que realizam simbiose, como *Rhizobium* e *Bradyrhizobium*, podem atuar formando nódulos nas raízes das plantas,

tais como, soja, ervilha, amendoim, alfafa, nas quais convertem N_2 em amônia e este pode ser usada pela planta como fonte N (Murray, 2011).

Dentro das células vegetais, os rizóbios passam por um processo de diferenciação que gera a forma especializada na fixação de N, o bacterióide. Então, um ou vários bacterióides são cercados por partes da membrana celular da planta para formar o chamado simbiossoma (Madigan e Martinko, 2006). Somente após a formação do simbiossoma, os bacterióides transformam o N atmosférico, através da enzima nitrogenase, em amônia. Em troca, a planta fornece ácidos orgânicos (para que os bacterióides produzam energia) e fornece um microambiente apropriado para a ação das nitrogenases, estabelecendo assim uma relação simbiótica entre planta e bactéria (Madigan e Martinko, 2006; Olanrewaju et al., 2017).

As bactérias de vida livre não interagem fisicamente com as raízes, elas vivem próximo das raízes, de modo que o N fixado por essas bactérias pode ser facilmente absorvido pelas plantas e estas podem se nutrir de exsudatos radiculares (aminoácidos, peptídeos, proteínas, enzimas, vitaminas e hormônios) (Tabassum et al., 2017).

Alguns exemplos de bactérias fixadores de N de vida livre são *Azotobacter*, *Azospirillum*, *Herbaspirillum*, *Burkholderia*, *Bacillus* e *Paenibacillus* (Huang et al., 2012; Angus et al., 2013; Anand et al., 2013; Habibi et al., 2014; Geddes et al., 2015; Goswami et al., 2016).

2.2.1.3 Solubilização de fósforo

O fósforo (P) é outro nutriente essencial para as plantas e desempenha um papel importante em quase todos os principais processos metabólicos, incluindo transferência de energia, transdução de sinal, respiração, biossíntese macromolecular e fotossíntese (Anand et al., 2016). E embora a reserva de P nos solos seja grande, ele é encontrado principalmente na forma de compostos insolúveis, que não podem ser absorvidos pelas plantas, limitando seu crescimento. As plantas absorvem fosfato

apenas como íons monobásicos ($H_2PO_4^-$) e dibásicos (HPO_4^{-2}) (Pérez-Montaño et al., 2014).

A solubilização e a mineralização do P por bactérias solubilizadoras de fosfato é uma característica importante. Os microrganismos desempenham um papel significativo na transformação de P no solo, incluindo a solubilização do P necessário para o crescimento das plantas (Rodríguez e Fraga, 1999).

O principal mecanismo de solubilização P inorgânico é baseado na secreção de ácidos orgânicos de baixo peso molecular pelos microrganismos, devido ao metabolismo do açúcar e os organismos residentes na rizosfera utilizam açúcares de exsudatos radiculares (Goswami et al., 2014; Sharma et al., 2013). Esses ácidos liberados pelos microrganismos atuam como bons quelantes de cátions divalentes de Ca^{2+} que acompanham a liberação de fosfatos de compostos fosfáticos insolúveis. Muitos dos microrganismos solubilizantes de fosfato diminuem o pH do meio por secreção de ácidos orgânicos, como ácidos acético, lático, málico, succínico, tartárico, glucônico, 2-cetoglucônico, oxálico e cítrico (Rodríguez e Fraga, 1999; Patel et al., 2015).

A solubilização orgânica do P também é chamada de mineralização do P orgânico. A mineralização do P orgânico do solo desempenha um papel imprescindível na ciclagem de fósforo de um sistema agrícola (Khan et al., 2007). Esse P pode ser liberado de compostos orgânicos no solo por enzimas tais como, fosfatases ácidas não específicas, fitases e fosfonatases e C-P liases (Sharma et al., 2013).

Os gêneros *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Microbacterium, Pseudomonas, Erwinia, Rhizobium, Mesorhizobium, Flavobacterium, Rhodococcus* e *Serratiae* são alguns que possuem a habilidade de solubilizar e mineralizar fosfatos (Oteino et al., 2015).

2.2.1.4 Solubilização de potássio

O potássio (K) é o terceiro macronutriente essencial para o crescimento das plantas e este elemento desempenha um papel vital em vários processos fisiológicos

e metabólicos da planta (Zhao et al., 2001), incluindo fotossíntese (Wang et al., 2012), crescimento vegetal, metabolismo, taxa de assimilação, acúmulo de açúcares, crescimento e desenvolvimento geral das plantas (Sparks e Huang, 1985). Como mais de 90% do K existe na forma de minerais insolúveis de rocha e silicato, a concentração de K solúvel é geralmente muito baixa no solo (Parmar e Sindhu, 2013).

Bactérias solubilizadoras de K, tais como *Acidothiobacillus* sp., *Bacillus edaphicus*, *Bacillus mucilaginosus*, *Pseudomonas* sp., *Burkholderia* sp. e *Paenibacillus* sp., foram relatadas pela sua ação de solubilizar K em formas assimiláveis, a partir de minerais de K no solo (Liu et al., 2012).

Como ocorre no caso da solubilização de P, o principal mecanismo da solubilização de K é a produção de ácidos orgânicos e inorgânicos e a produção de prótons (mecanismo de acidólise) (Sheng et al., 2008; Parmar e Sindhu, 2013; Maurya et al., 2014; Meena et al., 2015), que são capazes de converter o K insolúvel (mica, muscovita e biotita feldspato) em formas solúveis de K, facilmente absorvidas pela planta (Hu et al., 2006; Mo e Lian, 2011).

Entre os diferentes ácidos orgânicos envolvidos na solubilização do K insolúvel, ácido tartárico, ácido cítrico, ácido succínico, ácido α-cetoglucônico e ácido oxálico são os mais importantes liberados por bactérias solubilizadoras de K (Meena et al., 2014).

2.2.2 Mecanismos indiretos

A ação indireta dos microrganismos para promover o crescimento vegetal inclui a produção de agentes de biocontrole que inativam ou matam patógenos vegetais, proporcionando um ambiente saudável para as plantas (Naik et al., 2019).

Antibiose, competição, produção de enzimas líticas (quitinases e glucanases) com a capacidade de hidrolisar a parede celular fúngica, são considerados mecanismos indiretos de promoção de crescimento (Bhattacharyya e Jha, 2012). As bactérias podem também, indiretamente, melhorar o crescimento das plantas através

da supressão de patógenos e do aumento da imunidade inata da planta contra patógeno (Tabassum et al., 2017) (Figura 3).

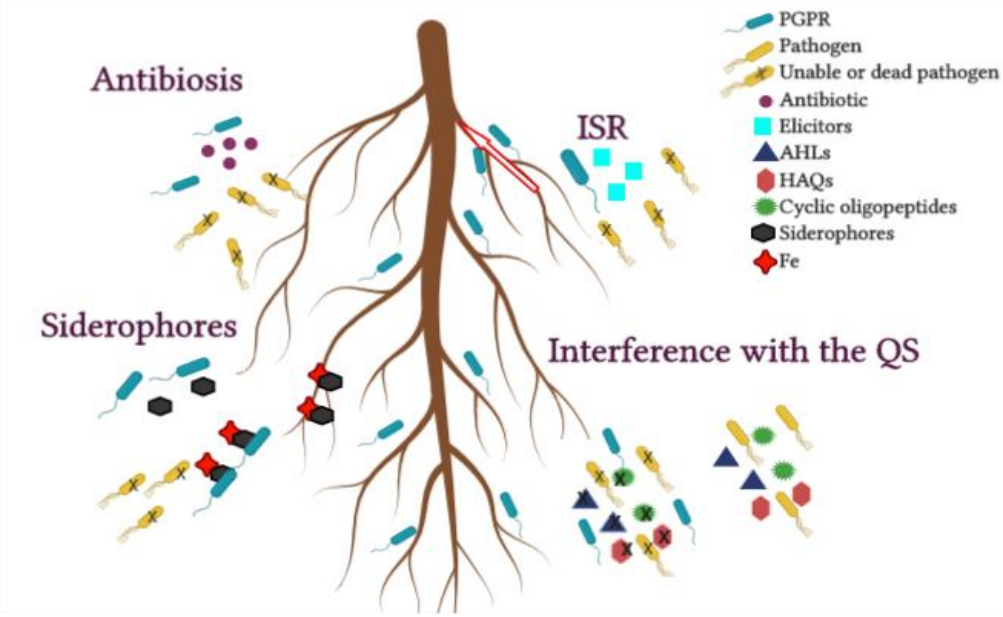

Figura 3. Mecanismos indiretos que beneficiam o crescimento das plantas partir das interações RPCP-hospedeiro, dentre eles, antibiose, produção de sideróforos, interferência com o sistema *Quorum Sensing* (QS), resistência sistêmica induzida (ISR) (Santos et al., 2020).

2.2.2.1 Produção de antibióticos

Antibióticos são toxinas naturais ou sintéticas de baixo peso que podem eliminar ou reduzir o crescimento bacteriano (Bakker et al., 2013). Vários antibióticos foram identificados como sendo produzidos por determinadas bactérias rizosféricas. Estes incluem anfisinas, fenazinas, 2-4-diacetil floroglucinol, pioluteorina, pirrolnitrina, cianeto de hidrogênio (HCN), oomicinas, polimixina, circulina, colistina, tensina, tropolona e lipopeptídeos cíclicos (Maksimov et al., 2011; Pandya e Saraf, 2014; Wani e Khan, 2014; Sherathia et al., 2016).

Algumas bactérias podem produzir um único antibiótico, enquanto outras podem secretar diversas substâncias (Reimer e Bode, 2014). A disponibilidade de nutrientes e estímulos ambientais ao redor definem fortemente a síntese de antibióticos (Majed et al., 2016).

As rizobactérias do gênero *Bacillus* estão as mais importantes para a produção de antibióticos (Jayaprakashvel e Mathivanan, 2011). *B. subtilis* e *B. amyloliquefaciens* são descritos como produtores de uma grande variedade de antibióticos, incluindo subtilina e bacilisina (Leclere et al., 2005, Chang et al., 2007).

2.2.2.2 Resistência sistêmica induzida

A resistência sistêmica induzida (RSI) é descrita como uma capacidade defensiva aprimorada das plantas em resposta a vários patógenos, induzida por microrganismos benéficos presentes na rizosfera (Conrath et al., 2015) ou seja, um fenômeno no qual a interação de alguns microrganismos com as raízes resulta em resistência da planta a algumas bactérias, vírus e fungos patogênicos (Lugtenberg e Kamilova, 2009). A RSI pode ainda ser desencadeada por estímulos ambientais específicos que levam à potenciação da defesa inata da planta contra desafios bióticos e este estado alto de alerta permite que a planta responda mais rápido e mais forte contra ataques subsequentes de patógenos (Van Loon, 1997).

A RSI é estimulada por microrganismos não patogênicos e começa na raiz, estendendo-se até a parte aérea (Solano et al., 2008), iniciando os mecanismos de defesa das plantas, protegendo partes não expostas das plantas contra futuros ataques por microrganismos patogênicos e insetos herbívoros. Essa resposta de defesa depende da sinalização de etileno e ácido jasmônico na planta (Van Loon, 2007, Pieterse et al., 2014).

A RSI tem sido relatada como um dos mecanismos pelos quais as RPCP podem reduzir a ocorrência de algumas doenças de plantas, modulando as propriedades físicas e bioquímicas das plantas hospedeiras e consequentemente promovendo o crescimento das plantas (Pieterse et al., 2012).

Alguns dos mecanismos de defesa produzidos na RSI nas plantas incluem reforço da parede celular (Ahn et al., 2007), produção de metabólitos secundários (Choudhary et al., 2007) e acúmulo de enzimas relacionadas à defesa, tais como quitinases, glucanases, peroxidase, fenilalanina amônia liase e polifenol oxidase (Bhattacharyya e Jha, 2012).

P. fluorescens (Pieterse et al., 1996), *Burkholderia phytofirmans* (Compant et al., 2005), *B. pumilus* (Benhamou et al., 1996), *Bacillus cereus* (Conn et al., 2008), *Rhizobium leguminosarum*, *P. putida* e *Serratia marcescens* (Bhattacharyya e Jha, 2012) são exemplos de rizobactérias que podem realizar a RSI.

2.2.2.3 Produção de sideróforos

O ferro (Fe) é um dos elementos mais abundantes da Terra, porém muitas vezes não está disponível para assimilação direta por plantas e microrganismos, devido que, na natureza este ocorre principalmente como Fe^{3+} e geralmente está presente na forma de hidróxidos e oxi-hidróxidos insolúveis (Rajkumar et al., 2010).

Algumas bactérias, a fim de obter Fe para seu crescimento e desenvolvimento, sintetizam moléculas de ligação de Fe de baixo peso molecular, denominadas de sideróforos (Mhlongo et al., 2018; Shaikh e Sayyed, 2015). As bactérias produtoras de sideróforos podem estimular o crescimento das plantas diretamente, melhorando a nutrição do Fe das plantas ou indiretamente, inibindo a atividade de patógenos vegetais na rizosfera, limitando sua disponibilidade de Fe (Solano et al., 2008, Ma et al., 2011).

O modo de supressão à patógenos realizado a partir da produção de sideróforos acontece com a restrição da sobrevivência de patógenos através da inibição da nutrição do Fe pela quelação do Fe disponível (Chaiharn et al., 2009). Em outras palavras, a solubilização e a aquisição competitiva de Fe em condições limitantes, reduz sua disponibilidade a outros habitantes do solo e subsequentemente limitam seu crescimento (Haas e Defago, 2005).

Além do Fe, existem evidências indicando que os sideróforos formam compostos estáveis com outros metais potencialmente tóxicos como alumínio (Al), cádmio (Cd), cobre (Cu), chumbo (Pb) e zinco (Zn) (Gururani et al., 2012). Esse fenômeno é vantajoso para aliviar o estresse nas plantas causado por metais potencialmente tóxicos presentes em solos e não apenas por causa do aumento da disponibilidade de nutrientes minerais para as plantas (Ahemad e Kibret, 2014).

Os sideróforos bacterianos são classificados em classes, com base nos tipos de ligante e características básicas de grupos funcionais que se coordenam com o Fe. As principais classes incluem catecolatos, carboxilato e hidroxamatos (Crowley et al., 2006).

Diversas espécies bacterianas são capazes de produzir sideróforos, dentre elas, *Azotobacter* (Romero-Perdomo et al., 2017), *Azospirillum* (Banik et al., 2016), *Bacillus* (Kesaulya et al., 2018; Pourbabaee et al., 2018), *Dickeya* (Sandy e Butler, 2011), *Klebsiella* (Zhang et al., 2017; Bailey et al., 2018), *Nocardia* (Hoshino et al., 2011), *Pantoea* (Burbank et al., 2015; Soutar e Stavrinides, 2018), *Paenibacillus* (Liu et al., 2017), *Pseudomonas* (Baune et al., 2017; Deori et al., 2018; Pourbabaee et al., 2018) e *Streptomyces* (Gáll et al., 2016; Goudjal et al., 2016).

2.2.2.4 Interferência no sistema *Quorum Sensing*

Muitas bactérias dependem da comunicação química para reconhecer o ambiente e recuperar informações sobre as densidades populacionais. Consequentemente, múltiplas moléculas são liberadas, que sincronizam a expressão de genes, coordenam o comportamento através de um processo denominado *Quorum Sensing* (QS) e determinam as relações com espécies procarióticas (Ortiz-Castro e Lopez-Bucio, 2019). QS é considerado um traço social das bactérias (Parsek e Greenberg, 2005).

A comunicação entre células é mediada por pequenas moléculas de sinal difusível denominadas auto-indutores (Fuqua et al., 1994). Geralmente, a sinalização mediada por auto-indutores ocorre em alta densidade populacional, pois assim, os

microrganismos agem em comunidade com vantagens para toda a população de células, simulando um organismo multicelular (Ganin et al., 2009; Bai e Vai, 2011).

Em bactérias Gram-negativas, frequentemente são encontradas N-acil homoserina lactonas (AHLs) e ocasionalmente 4-hidroxi-2-alquilquinolonas (HAQ), e as bactérias Gram-positivas usam principalmente oligopeptídeos cíclicos. As AHLs são as moléculas auto-indutoras mais comuns, elas regulam a expressão de genes implicados na produção do fator de virulência ou formação de biofilme em vários patógenos vegetais (Quiñones et al., 2005). Muitas plantas são capazes de produzir moléculas que interferem especificamente nos sistemas QS de bactérias associadas a plantas e, em qualquer caso, dependendo se a bactéria é detectada como patógeno ou como microrganismo benéfico, a molécula melhora ou inibe os fenótipos regulados por QS (Pérez-Montaño et al., 2014).

2.2.2.5 Rizorremediação e controle de estresse

As plantas são frequentemente expostas a vários estresses ambientais, e o crescimento das plantas pode ser inibido por um grande número de estresses bióticos (insetos, bactérias, fungos e vírus) e abióticos (radiação, salinidade, temperatura, inundação, seca e contaminantes), resultando em impactos altamente negativos na sobrevivência e na biomassa vegetal (Islam et al., 2016).

A rizorremediação usa plantas e microrganismos para remover, destruir ou eliminar metais tóxicos de ambientes contaminados de forma eficiente e econômica (Ma et al., 2011).

A maioria dos metais são tóxicos para as plantas, mas algumas bactérias são capazes de neutralizar a toxicidade do metal, ligando-o a grupos funcionais carregados negativamente em toda a parede celular, o que fornece interações com íons positivos, em particular íons de metal, um fenômeno chamado biossorção de metal (Syed e Chinthala, 2015). Espécies microbianas com considerável resistência a metais apresentaram imobilização de metais tóxicos ou redução de sua concentração

quando adicionadas a solos contaminados, reduzindo sua toxicidade para a planta ou cultura (Wani e Khan, 2014).

2.3 Promoção de crescimento em milho

O milho (*Zea mays* L.), pertencente à família Poaceae, é uma das culturas de cereais mais importantes do mundo e fornece alimentos para diversas populações (Shah et al., 2016). De acordo com o (International Grains Council, 2019), o consumo global de milho deverá subir para novos picos de até 1,3% nos próximos anos (projeção até 2024), bem como, o uso do milho para alimentação animal deverá se expandir no período mencionado.

O milho está entre as três culturas principais do mundo, fornecendo quase metade da energia diária para a África e as Américas (FAOSTAT, 2020). A demanda por milho por populações crescentes exigirá aumentos na produção, sustentabilidade e resiliência dos sistemas agrícolas baseados em milho (Shiferaw et al., 2011).

Para manter o aumento de produtividade do milho é necessário um acréscimo na quantidade de fertilizantes utilizados, resultando em aumento nos gastos de produção e maior impacto negativo ao meio ambiente. As RPCP podem contribuir para uma produção do milho menos poluente e dispendiosa.

Muitos efeitos benéficos do uso de RPCP sobre o crescimento e a produção da cultura do milho foram documentados. Breedt et al. (2017) encontraram um aumento de rendimento variando de 24 a 34% usando *Paenibacillus alvei*, *Bacillus safensis*, *B. pumilus* e *Brevundimonas vesicularis*. Cassán et al. (2009) avaliaram o efeito da mistura de *A. brasilense* com *Bradyrhizobium japonicum* e verificaram o aumento da taxa de germinação e desenvolvimento inicial das sementes.

Kuan et al. (2016) relataram que RPCP podem fornecer uma alternativa biológica para fixar o N_2 atmosférico e atrasar a remobilização de N na planta de milho, assim promovendo o aumento do rendimento da cultura com base na remobilização de N nas plantas. Este fato está diretamente correlacionado com aumento de produção de espigas em até 30,9% com entrada reduzida de N-fertilizante. Di Salvo

et al. (2018) relataram que RPCP usadas como inoculante de safras de cereais, incluindo milho, pode melhorar seu crescimento e rendimento de grãos. As respostas das culturas à inoculação são complexas porque são definidas por interações planta-microrganismos, muitas delas ainda desconhecidas. Assim, é necessário aprimorar o conhecimento sobre a ecologia microbiana da rizosfera de culturas sob diferentes práticas agrícolas.

Várias bactérias têm a capacidade de produzir AIA e têm efeitos positivos no aumento de massa de parte aérea e de raízes e na absorção de nutrientes pelas plantas de milho. Uma cepa de *B. subtilis* solubilizadora de fosfato e produtora de AIA proporcionou incrementos de produtividade na cultura do milho em condições de campo (Lobo et al., 2019).

O papel bioprotetor das RPCP nas lavouras de milho também foi estudado. O fungo toxigênico *Fusarium* é um dos gêneros importantes associados ao milho. Algumas RPCP, como *B. amyloliquefaciens* e *Microbacterium oleovorans*, foram capazes de proteger o milho contra *Fusarium verticillioides*, quando aplicados na forma de cobertura de sementes (Pereira et al., 2011). Além disso, algumas espécies de RPCP parecem promover o crescimento das plantas agindo como biofertilizantes e agentes de biocontrole. Por exemplo, cepas de *Burkholderia cepacia* foram observadas com características de biocontrole contra *Fusarium* spp. Simultaneamente, essas cepas também podem estimular o crescimento do milho em condições pobres em Fe por meio da produção de sideróforos (Bevivino et al., 1998).

2.4 Promoção de crescimento em cana-de-açúcar

A cana-de-açúcar (híbrido de espécies de *Saccharum*) é uma das culturas agrícolas mais antigas e valiosas do mundo, devido aos vastos benefícios associados ao seu uso industrial. A cana-de-açúcar é cultivada em regiões tropicais e subtropicais (Chhabra et al., 2016) e é usada principalmente para fornecer matéria-prima para as indústrias açucareiras para a produção de açúcar (Zhao e Li, 2015). Além disso, a

cana-de-açúcar também é vista com importância global devido aos benefícios associados à produção de biocombustíveis e biogás (Hoang et al., 2015).

Novas tecnologias desenvolvidas em todos os setores produtivos permitiram prolongar o período de plantio da cana-de-açúcar e, consequentemente, um uso mais eficiente de mão-de-obra e insumos, aumentando assim a sustentabilidade e a competitividade da indústria sucroalcooleira (Oliveira et al., 2012).

Uma das maiores limitações para a produção de cana-de-açúcar é o solo. Solos pobres e inadequados não atendem às necessidades nutricionais e de crescimento da cultura, portanto, esta não consegue atingir uma alta produção (Caione et al., 2015).

O uso das RPCP é uma alternativa promissora na produção de cana-de-açúcar com baixo impacto ambiental para aumentar a eficiência do uso de fertilizantes minerais incluindo fosfato, proporcionando altos rendimentos econômicos (Spolaor et al., 2016). Vários estudos de campo e casa de vegetação com fertilizantes fosfatados na cana-de-açúcar demonstram a importância das RPCP na disponibilização de P para essas plantas (Calheiros et al., 2012; Caione et al., 2015; Albuquerque et al., 2016; Borges et al., 2019).

Rosa et al. (2020) avaliaram o efeito da inoculação com três espécies de RPCP e cinco doses de P na cana-de-açúcar e relataram que a inoculação pode desempenhar um papel fundamental no cultivo, gerando grandes benefícios à cultura e reduzindo custos com fertilizantes para os produtores. Esses resultados revelaram que a combinação de *A. brasilense e B. subtilis* aliada ao baixo custo do P_2O_5 foi o melhor manejo de fertilizantes na cana-de-açúcar, o que é uma prática significativa de produção de cana-de-açúcar.

Santos et al. (2018) relatam que o uso de *B. subtilis* em conjunto com subprodutos na cultura canavieira pode melhorar os parâmetros de fertilidade do solo e diminuir os efeitos adversos associados à fertilização com vinhaça, além de proporcionar crescimento de parte aérea e raízes e proporcionar sinergia para alto rendimento da produção de cana-de-açúcar. Moura et al. (2018) mostraram que o uso de *Azospirillum* sp. na cultura da cana-de-açúcar melhorou o sistema radicular levando a uma melhor absorção de água e nutrientes que, por sua vez, podem influenciar positivamente na produtividade. Este relatório mostrou que a interação

significativa de cultivo, regime de água e inoculação de *Azospirillum* sp. sugere uma interação complexa desses fatores, da mesma forma, envolvendo o *pool* de auxinas nas plantas.

Li et al. (2017) isolaram *Pseudomonas* sp. associada à rizosfera de cana-de-açúcar e verificaram habilidades de promoção de crescimento do isolado, como solubilização de fosfato, produção de sideróforos, atividade da ACC-deaminase e produção de AIA, bem como atividade de fixação de N_2 e gerenciamento de doenças. Essas características são medidas como importantes características de promoção de crescimento e foram consideradas eficazes na melhoria do crescimento e do teor de N das plantas de cana-de-açúcar. A associação da RPCP na produção de cana-de-açúcar pode ser uma aplicação eminente de desenvolvimento de biofertilizantes, para a produção de culturas sustentáveis, na redução da poluição ambiental e no agronegócio biológico. Muthukumarasamy et al. (2017) verificaram que a associação entre a bactéria diazotrófica *Rosneateles terrae* e a solubilizante de P e K, *Burkholderia gladioli* com cana-de-açúcar foi capaz de aumentar a clorofila foliar, o teor de N e a biomassa total e incentivar os agricultores a usar RPCP para melhorar a disponibilidade de N, P e K no solo.

2.5 Identificação bacteriana através do gene 16S rRNA

Em microbiologia, os rRNA (RNA ribossômicos) são considerados as moléculas mais apropriadas para estudos de diversidade microbiana (Reis Junior et al., 2006). Dentre os principais benefícios, se destaca que, os genes de rRNA se encontram universalmente distribuídos e conservam-se em toda a sua extensão e estrutura (Reis Junior et al., 2006).

Para a classificação de microrganismos, é utilizada a análise comparativa da sequência de determinados genes de macromoléculas conservadas, como o rRNA. O sequenciamento do gene 16S rRNA tem sido amplamente usado para realização de análises taxonômicas e filogenéticas e é considerado o método de referência para a identificação bacteriana (Nolte e Caliendo, 2003. Becker et al., 2004). A utilização

desse gene inovou a ecologia microbiana e, com seu uso, pode-se averiguar e determinar posições filogenéticas de comunidades bacterianas presente no meio ambiente (Hentschel et al., 2002).

O gene 16S rRNA, assim como outros genes marcadores permitiram tanto identificar microrganismos por similaridade entre sequências, tal como agrupá-los num contexto filogenético. Esses avanços permitiram identificar, inclusive, filos inteiros que não possuem representantes cultiváveis (Garza e Dutilh, 2015). Além disso, árvores filogenéticas criadas a partir de sequências de 16S rRNA se assemelhavam muito àquelas criadas com base no conteúdo gênico total (Konstantinidis e Tiedje, 2005).

3. Referências

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. **Journal of King Saud University** 26: 1–20.

Ahmed E, Holmström SJM (2015) Microbe-mineral interactions: the impact of surface attachment on mineral weathering and element selectivity by microorganisms. **Chemical Geology** 403: 13–23.

Ahmad F, Ahmad I, Aqil F, Ahmed Wani A, Sousche YS (2016) Plant growth promoting potential of free-living diazotrophs and other rhizobacteria isolated from Northern Indian soil. **Biotechnology Journal** 1:1112–1123.

Ahn IP, Kim S, Lee YH, Suh SC (2007) Vitamin B-1-induced priming is dependent on hydrogen peroxide and the NPR1 gene in *Arabidopsis*. **Plant Physiology** 143: 838–848.

Albareda M, Dardanelli MS, Sousa C, Megías M, Temprano F, Rodríguez-Navarro DN (2006) Factors affecting the attachment of rhizospheric bacteria to bean and soybean roots. **FEMS Microbiology Letters** 259: 67-73.

Albuquerque AWD, Sá LDA, Rodrigues WA, Moura AB, Oliveira Filho MDS (2016) Growth and yield of sugarcane as a function of phosphorus doses and forms of application. **Revista Brasileira de Engenharia Agrícola e Ambiental** 20: 29–35.

Aloo BN, Makumba BA, Mbega ER (2019) The potential of Bacilli rhizobacteria for sustainable crop production and environmental sustainability. **Microbiological Research** 219: 26–39.

Anand K, Kumari B, Mallick MA (2016) Phosphate solubilizing microbes: an effective and alternative approach as biofertilizers**. International Journal of Pharmacy and Pharmaceutical Sciences** 8:37–40.

Anand R, Grayston S, Chanway C (2013) N_2-fixation and seedling growth promotion of lodgepole pine by endophytic *Paenibacillus polymyxa*. **Microbial Ecology** 66: 369–374.

Angus AA, Lee A, et al. (2013) Nodulation and effective nitrogen fixation of *Macroptilium atropurpureum* (siratro) by *Burkholderia tuberum*, a nodulating and plant growth promoting beta-proteobacterium, are influenced by environmental factors. **Plant Soil** 369: 543–562.

Babu AG, Kim JD, Oh BT (2013) Enhancement of heavy metal phytoremediation by *Alnus firma* with endophytic *Bacillus thuringiensis* GDB-1. **Journal of Hazardous Materials** 250–251: 477–483.

Bai AJ, Rai VR (2011) Bacterial quorum sensing and food industry. **Comprehensive Reviews in Food Science and Food Safety** 10: 184–194.

Bailey DC, Alexander E, Bailey DC, Alexander E, Rice MR, Drake EJ, Mydy LS, Aldrich CC, Gulick AM (2018) Structural and functional delineation of aerobactin biosynthesis in hypervirulent *Klebsiella pneumoniae*. **Journal of Biological Chemistry** 293: 7841–7852.

Bakker P, Berendsen RL, Doornbos RF, Wintermans PCA, Pieterse CMJ (2013) The rhizosphere revisited: root microbiomics. **Frontiers in Plant Science** 4: 1-7.

Banik A, Mukhopadhaya SK, Dangar TK (2016) Characterization of N_2-fixing plant growth promoting endophytic and epiphytic bacterial community of Indian cultivated and wild rice (*Oryza* spp.) genotypes. **Planta** 243: 799–812.

Bashan Y, Holguin G (1997) *Azospirillum*-plant relationships: environmental and physiological advances (1990-1996). **Canadian Journal of Microbiology** 43:103–121.

Baune M, Qi YL, Scholz K, Volmer DA, Hayen H (2017) Structural characterization of pyoverdines produced by *Pseudomonas putida* KT2440 and *Pseudomonas taiwanensis* VLB120. **BioMetals** 30: 589–597.

Belimov AA, Dodd IC, Safronova VI, Dumova VA, Shaposhnikov AI, Ladatko AG, Davies WJ (2014) Abscisic acid metabolizing rhizobacteria decrease ABA concentrations in planta and alter plant growth. **Plant Physiology and Biochemistry** 74: 84–91.

Belimov AA, Safronova VI, et al. (2001) Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. **Canadian Journal of Microbiology** 47: 642–652.

Benhamou N, Kloepper JW, Hallman A, Tuzun S (1996) Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. **Plant Physiology** 112: 919–929.

Bevivino A, Sarrocco S, Dalmastri C, Tabacchioni S, Cantale C, Chiarini L (1998) Characterization of a free-living maize-rhizosphere population of *Burkholderia cepacia*: Effect of seed treatment on disease suppression and growth promotion of maize. **FEMS Microbiology Ecology** 27:225–237.

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. **World Journal of Microbiology and Biotechnology** 28: 1327–1350.

Borges BMMN, Abdala DB, Souza MFS, Viglio LM, Coelho MJA, Pavinato OS, Franco, HCJ (2019) Organomineral phosphate fertilizer from sugarcane by product and its effects on soil phosphorus availability and sugarcane yield. **Geoderma** 339: 20–30.

Breedt G, Labuschagne N, Coutinho TA (2017) Seed treatment with selected plant growth-promoting rhizobacteria increases maize yield in the field. **Annals of Applied Biology** 171: 229–236.

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. **Annual Review of Plant Biology** 64: 807–838.

Burbank L, Mohammadi M, Roper MC (2015) Siderophore-mediated iron acquisition influences motility and is required for full virulence of the xylem-dwelling bacterial Phytopathogen *Pantoea stewartii* subsp. *stewarti*i. **Applied and Environmental Microbiology** 81: 139–148.

Caione G, Prado RDM, Campos CNS, Rosatto Moda L, de Lima Vasconcelos R, Pizauro Júnior JM (2015) Response of sugarcane in a red ultisol to phosphorus rates, phosphorus sources, and filter cake. **The Scientific World Journal** 2015: 1–10.

Calheiros AS, de Oliveira MW, Ferreira VM, de Souza Barbosa GV, Santiago AD, dos Santos Aristides EV (2012) Production of biomass, from sugar and protein in function of sugarcane varieties and phosphorous fertilization. **Semina Ciencias Agrarias** 33: 809–818.

Camilios-Neto D, Bonato P, et al. (2014) Dual RNA-seq transcriptional analysis of wheat roots colonized by *Azospirillum brasilense* reveals up-regulation of nutrient acquisition and cell cycle genes. **BMC Genomics** 15: 1-13.

Cassán F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V (2009) *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). **European Journal of Soil Biology** 45, 28–35.

Chaiharn M, Chunhaleuchanon S, Lumyong S (2009) Screening siderophore producing bacteria as potential biological control agent for fungal rice pathogens in Thailand. **World Journal of Microbiology and Biotechnology** 25: 1919–1928.

Chang WT, Chen, YC, Jao CL (2007) Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes. **Bioresource Technology** 98: 1224–1230.

Cheng WH, Endo A, et al. (2002) A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. **Plant Cell** 14: 2723–2743.

Chhabra ML, Parameswari B, Viswanathan R (2016) Pathogenic behaviour pattern of *Colletotrichum falcatum* isolates of sugarcane in sub-tropical India. **Vegetos** 29:76.

Choudhary DK, Prakash A, Johr BN (2007) Induced systemic resistance (ISR) in plants: mechanism of actionIndian. **Journal of Microbiology** 47: 289–297.

Cohen AC, Travaglia CN, Bottini R, Piccoli PN (2009) Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. **Botany** 87: 455–462.

Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. **Applied and Environmental Microbiology** 71: 4951–4959.

Conn VM, Walker AR, Franco CMM (2008) Endophytic actinobacteria induce defense pathways in *Arabidopsis thaliana*. **Molecular Plant-Microbe Interactions** 21: 208–218.

Conrath U, Beckers GJM, Langenbach CJG, Jaskiewicz MR (2015) Priming for Enhanced Defense. In.: VanAlfen NK (Eds.) **Annual review of phytopathology**. p. 97-119.

Cortleven A, Schmulling T (2015) Regulation of chloroplast development and function by cytokinin**. Journal of Experimental Botany** 66: 4999–5013.

Crowley DE (2006) Microbial siderophores in the plant rhizosphere. In.: Barton LL, Abadía J (Eds.) **Iron nutrition in plants and rhizospheric microorganisms**. Dordrecht: Springer p. 169–198.

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. In.: **food security in nutrient-stressed environments: exploiting plants' genetic capabilities**. Netherlands: Springer p. 201–213.

de Salamone IEG, Hynes RK, Nelson LM (2001) Cytokinin production by plant growth promoting rhizobacteria and selected mutants. **Canadian Journal of Microbiology** 47: 404–411.

Deori M, Jayamohan NS, Kumudini BS (2018) Production, characterization and iron binding affinity of hydroxamate siderophores from rhizosphere associated fluorescent *Pseudomonas*. **Journal of Plant Protection Research** 58: 36–43.

Desbrosses G, Contesto C, Varoquaux F, Galland M, Touraine B (2009) PGPR-*Arabidopsis* interactions is a useful system to study signaling pathways involved in plant developmental control. **Plant Signaling & Behavior** 4: 319-321.

Di Salvo LP, Cellucci GC, Carlino ME, de Salamone IEG (2018) Plant growth-promoting rhizobacteria inoculation and nitrogen fertilization increase maize (*Zea mays* L.) grain yield and modified rhizosphere microbial communities. **Applied Soil Ecology** 126: 113–120.

Dini-Andreote F, Raaijmakers JM (2018) Embracing community ecology in plant microbiome research. **Trends Plant Sci** 23: 467-469.

Dodd IC (2007) Soil moisture heterogeneity during deficit irrigation alters root-to-shoot signalling of abscisic acid. **Functional Plant Biology** 34: 439–448.

Dodd IC, Zinovkina NY, Safronova, VI, Belimov AA. (2010) Rhizobacterial mediation of plant hormone status. **Annals of Applied Biology** 157: 361–379.

Dubey RC, Maheshwari DK, Kumar V, Pandey, RR (2012) Growth enhancement of *Sesamum indicum* L. by rhizosphere-competent *Azotobacter chroococcum* AZO2 and its antagonistic activity against *Macrophomina phaseolina*. **Archives of Phytopathology and Plant Protection** 45: 437–454.

Duca D, Lorv J, Patten CL, Rose D, Glick BR (2014) Indole-3-acetic acid in plant-microbe interactions. **Antonie Van Leeuwenhoek** 106: 85–125.

FAOSTAT Food Balance Sheets (2020) Disponível em: <http://www.fao.org/faostat/en/#data/FBS>. Acesso em 24 abr. 2020

Farajzadeh D, Yakhchali B, Aliasgharzad N, Sokhandan-Bashir N, Farajzadeh M (2012) Plant growth promoting characterization of indigenous azotobacteria isolated from soils in Iran. **Current Microbiology** 64: 397–403.

Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G (2007) Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture médium. **Applied Microbiology and Biotechnology** 76: 1145–11524.

Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria - the luxr-luxi family of cell density-responsive transcriptional regulators. **Journal of Bacteriology** 176: 269–275.

Garza DR, Dutilh BE (2015) From cultured to uncultured genome sequences: Metagenomics and modeling microbial ecosystems. Springer p: 4287–4308.

Ganin H, Tang X, Meijler MM (2009) Inhibition of *Pseudomonas aeruginosa* quorum sensing by AI-2 analogs. **Bioorganic & Medicinal Chemistry Letters** 19: 3941–3944.

Gáll T, Lehoczki G, Gyémánt G, Emri T, Szigeti ZM, Balla G, Balla J, Pócsi I (2016) Optimization of desferrioxamine e production by *Streptomyces parvulus*. **Acta Microbiologica et Immunologica Hungarica** 63: 475–489.

Geddes BA, Ryu MH, Mus F, Costas AG, Peters JW, Voigt CA (2015) Use of plant colonizing bacteria as chassis for transfer of N_2-fixation to cereals. **Current Opinion in Biotechnology** 32: 216–222.

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. **Microbiological Research** 169: 30–39.

Gopalakrishnan S, Srinivas V, Samineni S (2017) Nitrogen fixation, plant growth and yield enhancements by diazotrophic growth-promoting bacteria in two cultivars of chickpea (*Cicer arietinum* L.). **Biocatalysis and Agricultural Biotechnology** 11: 116–123.

Goswami D, Dhandhukia P, Patel P, Thakker JN (2014) Screening of PGPR from saline desert of Kutch: growth promotion in *Arachis hypogea* by *Bacillus licheniformis* A2. **Microbiological Research** 169: 66–75.

Goswami D, Thakker JN, Dhandhukia PC (2016) Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. **Cogent Food & Agriculture** 2: 1-19.

Goudjal Y, Zamoum M, Meklat A, Sabaou N, Mathieu F, Zitouni A. (2016) Plant-growth-promoting potential of endosymbiotic actinobacteria isolated from sand truffles (*Terfezia leonis* Tul.) of the Algerian Sahara. **Annals of Microbiology** 66: 91–100.

Grosskinsky DK, Naseem M, et al. (2011) Cytokinins mediate resistance against pseudomonas syringae in tobacco through increased antimicrobial phytoalexin synthesis independent of salicylic acid signaling. **Plant Physiology** 157: 815–830.

Grossmann K (2010). Auxin herbicides: current status of mechanism and mode of action. **Pest Management Science** 66: 113–120.

Guo HY, Wang YC, Liu, HZ, Hu P, Jia YY, Zhang CR, Wang Y, Gu S, Yang C, Wang, C (2015) Exogenous GA_3 application enhances xylem development and induces the expression of secondary wall biosynthesis related genes in *Betula platyphylla*. **International Journal of Molecular Sciences** 16: 22960–22975.

Gururani MA, Upadhyaya CP, Baskar V, Venkatesh J, Nookaraju A, Park SW (2013) Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in solanum tuberosum through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. **Journal of Plant Growth Regulation** 32: 245–258.

Haas D, Defago G (2005) Biological control of soil-borne pathogens by *Fluorescent pseudomonads.* **Nature Reviews Microbiology** 3: 307–319.

Habibi S, Djedidi S, Prongjunthuek K, Mortuza MF, Ohkama-Ohtsu N, Sekimoto H, Yokoyoma T (2014) Physiological and genetic characterization of rice nitrogen fixer PGPR isolated from rhizosphere soils of different crops. **Plant Soil** 379: 51–66.

Halliday KJ, Martinez-Garcia JF, Josse EM (2009) Integration of light and auxin signaling **Cold Spring Harbor Perspectives in Biology** 1: 1-11.

Hartmann A, Schmid M, Van Tuinen D, Berg G (2009) Plant-driven selection of microbes. **Plant Soil** 321: 235-257.

Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J, Moore BS (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. **Applied and environmental microbiology** 68: 4431-4440.

Hoang NV, Furtado A Botha FC, Simmons BA, Henry RJ (2015) Potential for genetic improvement of sugarcane as a source of biomass for biofuels. **Frontiers in Bioengineering and Biotechnology** 3: 1-15.

Hoshino Y, Chiba K, Ishino K, Fukai T, Igarashi Y, Yazawa K, Mikami Y, Ishikawa J (2011) Identification of nocobactin NA biosynthetic gene clusters in *Nocardia farcinica*. **Journal of Bacteriology** 193: 441–448.

Hu XF, Chen JS, Guo JF (2006) Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. **World Journal of Microbiology and Biotechnology** 22: 983–990.

Huang CJ, Tsay JF, Chang SY, Yang HP, Wu WS, Chen CY (2012). Dimethyl disulfide is an induced systemic resistance elicitor produced by *Bacillus cereus* C1L. **Pest Management Science** 68: 1306–1310.

INTERNATIONAL GRAINS COUNCIL (2019) **Five-year baseline projections of supply and demand for wheat, maize (corn), rice and soyabeans to 2023/24**. London: International Grains Council p. 32.

Iqbal A, Hasnain S (2013) Auxin producing *Pseudomona*s strains: biological candidates to modulate the growth of *Triticum aestivum* beneficially. **American Journal of Plant Sciences** 4: 1693–1700.

Islam F, Yasmeen T, Ali Q, Mubin M, Ali S, Arif MS, Hussain S, Riaz M, Abbas F (2016) Copper-resistant bacteria reduces oxidative stress and uptake of copper in lentil plants: potential for bacterial bioremediation. **Environmental Science and Pollution Research** 23: 220–233.

Jayaprakashvel M, Mathivanan N (2011) Management of plant diseases by microbial metabolites. In.: Maheshwari DK (Eds.) **Bacteria in Agrobiology: Plant Nutrient Management**. Berlin: Springer p. 237–265.

Jha CK, Saraf M (2015) Plant growth promoting rhizobacteria (PGPR): a review. **Journal of Agricultural Research and Development** 2015: 108–119.

Joo GJ, Kim YM, Lee, IJ, Song KS, Rhee IK (2004) Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macroides* and *Bacillus pumilus*. **Biotechnology Letters** 26: 487–49.

Kamilova F, Kravchenko LV, Shaposhnikov AI, Azarova T, Makarova N, Lugtenberg B (2006) Organic acids, sugars and L-tryptophane in exudates of vegetables growing on stonewool and their effects on activities of rhizosphere bacteria. **Molecular Plant-Microbe Interactions** 9: 250–256.

Kamran MA, Eqani ASMAS, Bibi S, Xu RK, Monis MFH, Katsoyiannis A, Bokhari H, Chaudhary HJ (2016) Bioaccumulation of nickel by *E. sativa* and role of plant growth promoting rhizobacteria (PGPRs) under nickel stress. **Ecotoxicology and Environmental Safety** 126: 256–263.

Kaur H, Kaur J, Gera R (2016) Plant growth promoting rhizobacteria: a boon to agriculture. **International Journal of Cell Science and Biotechnology** 5: 17–22.

Kesaulya H, Hasinu JV, Tuhumury, GN (2018) Potential of *Bacillus* spp produces siderophores insuppressing thewilt disease of banana plants. In.: **IOP Conference Series: Earth and Environmental Science.** Semarang: IOP Publishing p: 1–6.

Khalid A, Tahir S, Arshad, M, Zahir, ZA (2004) Relative efficiency of rhizobacteria for auxin biosynthesis in rhizosphere and non-rhizosphere soils. **Australian Journal of Soil Research** 42: 921–926.

Khan AL, Waqas M, et al. (2014) Bacterial endophyte *Sphingomonas* sp LK11 produces gibberellins and IAA and promotes tomato plant growth. **Journal of Microbiology** 52: 689–695.

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. **Agronomy for sustainable development** 27: 29-43.

Kingston G (2013) Mineral nutrition of sugarcane. In.: Moore PH, Both FC (Eds.) **Sugarcane: Physiology, Biochemistry, and Functional Biology**. John Wiley & Sons p: 85–120.

Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In: **IV International Conference on Plant Pathogenic Bacteria.** p. 879-882.

Konstantinidis KT, Tiedje, JM (2005) Genomic insights that advance the species definition for prokaryotes. **Proceedings of the National Academy of Sciences of the United States of America, National Acad Sciences** 102: 2567–2572.

Krey T, Vassilev N, Baum C, Eichler-Löbermann B (2013). Effects of long-term phosphorus application and plant-growth promoting rhizobacteria on maize phosphorus nutrition under field conditions. **European Journal of Soil Biology** 55: 124–130.

Kuan KB, Othman R, Rahim KA, Shamsuddin ZH (2016) Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. **PLoS ONE** 11:19.

Kumar A, Maurya BR, Raghuwanshi R, Meena VS, Tofazzal Islam M (2017) Co-inoculation with *Enterobacter* and rhizobacteria on yield and nutrient uptake by wheat (*Triticum aestivum* L.) in the alluvial soil under Indo-Gangetic Plain of India. **Plant Growth Regulation** 36: 608–617.

Leclere V, Bechet M, Adam A, Guez JS, Wathelet B, Ongena M, Thonart P, Gancel F, Chollet-Imbert, M, Jacques P (2005) Mycosubtilin overproduction by *Bacillus subtilis* BBG100 enhances the organism's antagonistic and biocontrol activities. **Applied and Environmental Microbiology** 71: 4577–4584.

Lee CM, Monson RE, Adams RM, Salmond GP (2017) The LacI-family transcription factor, RbsR, is a pleiotropic regulator of motility, virulence, siderophore and antibiotic production, gas vesicle morphogenesis and flotation in Serratia. **Frontiers in Microbiology** 8: 1-14.

Li HB, Singh RK, Singh P, Song QQ, Xing YX, Yang LT, Li YA (2017) Genetic diversity of nitrogen-fixing and plant growth promoting pseudomonas species isolated from sugarcane rhizosphere. **Frontiers in Microbiology** 8:1-20.

Li Q, Saleh-Lakha S, Glick BR (2005) The effect of native and ACC deaminase-containing *Azospirillum brasilense* Cd1843 on the rooting of carnation cuttings. **Canadian Journal of Microbiology** 51: 511–514.

Liu D, Yang QQ, Ge K, Hu XN, Qi GZ, Du BH, Liu K, Ding Y (2017) Promotion of iron nutrition and growth on peanut by *Paenibacillus illinoisensis* and *Bacillus* sp strains in calcareous soil. **Brazilian Journal of Microbiology** 48: 656–670.

Liu, DF, LianB, Dong HL (2012) Isolation of *Paenibacillus* sp and assessment of its potential for enhancing mineral weathering. **Geomicrobiology Journal** 29: 413–421.

Liu FC, Xing SJ, Ma HL, Du ZY, Ma BY (2013) Cytokinin-producing, plant growth-promoting rhizobacteria that confer resistance to drought stress in *Platycladus orientalis* container seedlings. **Applied Microbiology and Biotechnology** 97: 9155–9164.

Lobo LLB, dos Santos RM, Rigobelo EC (2019) Promotion of maize growth using endophytic bacteria under greenhouse and field condition. **Australian Journal of Crop Science** 13: 2067–2074.

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. **Annual Review of Microbiology** 63: 541–556.

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. **Biotechnology Advances** 29: 248–258.

Madigan MT, Martinko JM (2006) **Biology of Microorganisms**. London: Pearson Prentice.

Majed R, Faille C, Kallassy M, Gohar M (2016) *Bacillus cereus* biofilms-same, only different. **Frontiers in Microbiology** 7:1-16.

Maksimov IV, Abizgil'dina RR, Pusenkova LI (2011) Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens (review). **Applied Biochemistry and Microbiology** 47: 333–345.

Mansfield TA, Hetherington AM, Atkinson CJ (1990) Some current aspects of stomatal physiology. **Annual Review of Plant Physiology and Plant Molecular Biology** 41: 55–75.

Martinez C, Espinosa-Ruiz, Prat S (2016) Gibberellins and plant vegetative growth **Annual Plant Reviews** 49: 285-322.

Maurya BR, Meena VS, Meena OP (2014) Influence of Inceptisol and Alfisol's potassium solubilizing bacteria (KSB) isolates on release of K from waste mica. **Vegetos** 27: 181–187.

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. **Plant Physiology and Biochemistry** 42: 565–572.

McSteen P (2010) Auxin and monocot development. **Cold Spring Harbor Perspectives in Biology** 3: 1-17.

Meena KK, Sorty AM, et al. (2017). Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. **Frontiers in Plant Science** 8:1-25.

Meena VS, Maurya BR, Verma JP (2014) Does a rhizospheric microorganism enhance K+ availability in agricultural soils? **Microbiological Research** 169: 337–347.

Meena VS, MauryaBR, Verma JP, Aeron A, Kumar A, Kim K, Bajpai VK (2015). Potassium solubilizing rhizobacteria (KSR): Isolation, identification, and K-release dynamics from waste mica. **Ecological Engineering** 81: 340–347.

Merino E, Jensen RA, Yanofsky C (2008) Evolution of bacterial *trp* operons and their regulation. **Current Opinion in Microbiology** 11: 78–86.

Mhlongo MI, Piater LA, Madala NE, Labuschagne N, Dubery IA (2018) The chemistry of plant-microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. **Frontiers in Plant Science** 9:1-17.

Mo B, Lian B (2011) Interactions between *Bacillus mucilaginosus* and silicate minerals (weathered adamellite and feldspar): Weathering rate, products, and reaction mechanisms. **Chinese Journal of Geochemistry** 30:187–192.

Moura RTDA, Garrido MDS, Sousa CDS, Menezes RSC, Sampaio EVDSB (2018). Comparison of methods to quantify soil microbial biomass carbon. **Acta Scientiarum Agronomy** 40: 1-7.

Munemasa S, Hauser F, Park J, Waadt R, Brandt B, Schroeder JI (2015) Mechanisms of abscisic acid-mediated control of stomata! aperture. **Current Opinion in Plant Biology** 28: 154–162.

Muñoz-Rojas J, Caballero-Mellado J (2003) Population dynamics of Gluconacetobacter diazotrophicus in sugarcane cultivars and its effect on plant growth. **Microbial ecology** 46: 454-464.

Murray JD (2011) Invasion by invitation: rhizobial infection in legumes. **Molecular Plant-Microbe Interactions** 24: 631–639.

Muthukumarasamy R, Revathi G, Vadivelu M, Aruri K (2017) Isolation of bacterial strains possessing nitrogen-fixation, phosphate and potassium-solubilization and their inoculation effects on sugarcane**. Indian Journal of Experimental Biology** 55: 161–170.

Naik K, Mishra S, Srichandan H, Singh PK, Sarangi PK (2019) Plant growth promoting microbes: potential link to sustainable agriculture and environment. **Biocatalysis and Agricultural Biotechnology** 21:101326.

Nolte FS, Caliendo AM (2003) Molecular detection and identification of microorganisms In.: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (Eds.) **Manual of Clinical Microbiology.** Washington: ASM Press, p.234-256.

O'Brien JA, Benkova E (2013) Cytokinin cross-talking during biotic and abiotic stress responses. **Frontiers in Plant Science** 4: 1-11.

Olanrewaju OS, Glick BR, Babalola OO (2017) Mechanisms of action of plant growth promoting bacteria. **World Journal of Microbiology and Biotechnology** 33:1-16.

Oliveira TBA, Selig PM, Barbosa VM, de Souza Campos LM, Bornia AC, de Oliveira MW (2012) Tecnologia e custos de produção de cana-de-açúcar: um estudo de caso em uma propriedade agrícola. **Latin American Journal of Business Management** 3: 151–172.

Ortiz-Castro R, Lopez-Bucio J (2019) Review: Phytostimulation and root architectural responses to quorum-sensing signals and related molecules from rhizobacteria. **Plant Science** 284: 135–142.

Oteino N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, David N, Dowling DN (2015) Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas isolates*. **Frontiers in Microbiology** 6:1-9.

Pandya U, Saraf M (2014) *In vitro* evaluation of PGPR strains for their biocontrol potential against fungal pathogens. In.: Kharwar RN et al. (Eds.) **Microbial Diversity and Biotechnology in Food Security**. New Delhi: Springer, p. 293–305.

Parmar P, Sindhu SS (2013) Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. **Journal of Microbiology Research** 3: 25–31.

Parsek MR, Greenberg EP (2005) Sociomicrobiology: the connections between quorum sensing and biofilms. **Trends in microbiology** 13: 27-33.

Patel K, Goswami D, Dhandhukia P, Thakker J (2015) Techniques to study microbial phytohormones. In.: Maheshwari DK (Eds.) **Bacterial Metabolites in Sustainable Agroecosystem**. Dordrecht: Springer, p: 1–27.

Patten CL, Glick BR (1996) Bacterial biosynthesis on indole-3-acetic acid. **Canadian Journal of Microbiology** 42: 207–220.

Pedrinho EAN, Júnior RFG, Campanharo JC, Alves LMC, de Macedo Lemos EG (2010) Identificação e avaliação de rizobactérias isoladas de raízes de milho. **Bragantia** 69: 905-911.

Pereira P, Ibàñez F, Rosenblueth M, Etcheverry M, Martínez-Romero E (2011) Analysis of the bacterial diversity associated with the roots of maize (*Zea mays* L.) through culture-dependent and culture-independent methods. **International Scholarly Research Notices** 2011:1-10.

Pérez-Montaño F, Jiménez-Guerrero I, Sánchez-Matamoros RC, López-Baena FJ, Ollero FJ, Rodríguez-Carvajal MA, Bellogína RA, Rosario Espunya M (2013) Rice and bean AHL-mimic quorum-sensing signals specifically interfere with the capacity to form biofilms by plant-associated bacteria **Research in Microbiology** 164: 749–760.

Perez-Montano F, Alias-Villegas C, Bellogin RA, del Cerro P, Espuny MR, Jimenez-Guerrero I, López-Baena J, Ollero FJ, Cubo T (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. **Microbiological Research** 169: 325–336.

Phillips KA, Skirpan AL, Liu X, Christensen A, Slewinski TL, Hudson C, Barazesh S, Cohen JD, Malcomber S, McSteen P (2011). vanishing tassel2 encodes a grass-specific tryptophan aminotransferase required for vegetative and reproductive development in maize. **Plant Cell** 23: 550–566.

Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM (2012) Hormonal modulation of plant immunity. In.: Schekman R (Eds.) **Annual Review of Cell and Developmental Biology**. Palo Alto: Annual Reviews, p: 489–521.

Pieterse CMJ, vanWees SCM, Hoffland E, vanPelt JA, vanLoon LC (1996) Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression**. Plant Cell** 8: 1225–1237.

Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker P (2014) Induced systemic resistance by beneficial microbes. In.: VanAlfen NK (Eds.) **Annual Review of Phytopathology**. Palo Alto: Annual Reviews, p. 347–375.

Porcel R, Zamarreno AM, Garcia-Mina JM, Aroca R (2014) Involvement of plant endogenous ABA in *Bacillus megaterium* PGPR activity in tomato plants. **BMC Plant Biology** 14: 1-12.

Pospisilova J (2003) Participation of phytohormones in the stomatal regulation of gas exchange during water stress. **Biologia Plantarum** 46: 491–506.

Poupin MJ, Greve M, Carmona V, Pinedo I (2016) A complex molecular interplay of auxin and ethylene signaling pathways is involved in *Arabidopsis* growth promotion by *Burkholderia phytofirmans* PsJN. **Frontiers in Plant Science** 7:1-16.

Poupin MJ, Timmermann T, Vega A, Zuniga A, Gonzalez B (2013) Effects of the plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN throughout the life cycle of *Arabidopsis thaliana*. **PLoS ONE** 8: 1-15.

Pourbabaee AA, Shoaibi F, Emami S, Alikhani HA (2018) The potential contribution of siderophore producing bacteria on growth and Fe ion concentration of sunflower (*Helianthus annuus* L.) under water stress. **Journal of Plant Nutrition** 41: 619–626.

Quiñones B, Dulla G, Lindow SE (2005) Quorum sensing regulates exopolysaccharide production, motility, and virulence in *Pseudomonas syringae*. **Molecular Plant-Microbe Interactions** 18: 682–693.

Raj D, Antil RS (2011) Evaluation of maturity and stability parameters of composts prepared from agro-industrial wastes. **Bioresource technology** 102: 2868–2873.

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. **Trends in Biotechnology** 28: 142–149.

Reed SC, Cleveland CC, Townsend AR (2011) Functional ecology of free-living nitrogen fixation: a contemporary perspective. In.: Futuyma DJ, Shaffer HB, Simberloff D (Eds.) **Annual Review of Ecology, Evolution, and Systematics**. Palo Alto: Annual Reviews, p: 489–512.

Reid MS (1988) The role of ethylene in flower senescence. In.: IV International Symposium on Postharvest Physiology of Ornamental Plants. **Resumos...** p 261.

Reimer D, Bode HB (2014) A natural prodrug activation mechanism in the biosynthesis of nonribosomal peptides. **Natural Product Reports** 31: 154–159.

Reis Junior FB, Reis VM, Teixeira KRS (2006) Restrição do 16S-23S DNAr intergênico para avaliação da diversidade de *Azospirillum amazonense* isolado de *Brachiaria* spp. **Pesquisa Agropecuária Brasileira** 41: 431-438.

Riggs PJ, Chelius MK, Iniguez AL, Kaeppler SM, Triplett EW (2001) Enhanced maize productivity by inoculation with diazotrophic bacteria**. Functional Plant Biology** 28: 829–836.

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. **Biotechnology Advances** 17: 319-339.

Romero-Perdomo F, Abril J, Camelo M, Moreno-Galvan A, Pastrana I, Rojas-Tapias D, Bonilla R (2017) *Azotobacter chroococcum* as a potentially useful bacterial biofertilizer for cotton (*Gossypium hirsutum*): effect in reducing N fertilization. **Revista Argentina de Microbiología** 49: 377–383.

Rosa PAL, Mortinho ES, Jalal A, Galindo FS, Buzetti S, Fernandes GC, Neto MB Pavinato OS, Teixeira Filho, MCM (2020) Inoculation with growth-promoting bacteria associated with the reduction of phosphate fertilization in sugarcane. **Frontiers in Environmental Science** 8:1-18.

Sah SK, Reddy KR, Li JX (2016) Abscisic acid and abiotic stress tolerance in crop plants. **Frontiers in Plant Science** 7: 1-26.

Saleem M, Zamir MSI, Haq I, Irshad MZ, Khan MK, Asim M, Zaman Q, Ali I, Khan A, Rehman S (2015). Yield and quality of forage oat (*Avena sativa* L.) cultivars as affected by seed inoculation with nitrogenous strains. **American Journal of Plant Sciences** 6: 3251-3259.

Sandhya V, Ali SZ, Grover M, Reddy G, Venkateswarlu B (2010) Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. **Plant Growth Regulation** 62: 21–30.

Sandy M, Butler A (2011) Chrysobactin siderophores produced by *Dickeya chrysanthemi* EC16. **Journal of Natural Products** 74: 1207–1212.

Santos RM, Kandasamy S, Rigobelo EC (2018) Sugarcane growth and nutrition levels are differentially affected by the application of PGPR and cane waste. **MicrobiologyOpen** 7:1-9.

Santos RM, Dias PA, Lobo LL, Rigobelo EC (2020). Growth-promoting rhizobacteria in maize and sugarcane. **Frontiers in Sustainable Food Systems** 4: 1-15.

Sattari SZ, Bouwman AF, Giller KE, van Ittersum MK (2012) Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. **Proceedings of the National Academy of Sciences of the United States of America** 109: 6348–6353.

Schaller GE, Bishopp A, Kieber JJ (2015) The Yin-Yang of hormones: cytokinin and auxin interactions in plant development. **Plant Cell** 27: 44–63.

Sgroy V, Cassán F, Masciarelli O, Del Papa MF, Lagares A, Luna V (2009) Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte *Prosopis strombulifera*. **Applied Microbiology and Biotechnology** 85: 371–381.

Shah TR, Prasad K, Kumar P (2016) Maize - A potential source of human nutrition and health: A review. **Cogent Food & Agriculture** 2: 1-9.

Shahzad R, Waqas M, Khan AL, Asaf S, Khan MA, Kang SM, Yun BW, Lee IJ (2016). Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of *Oryza sativa*. **Plant Physiology and Biochemistry** 106: 236–243.

Shaikh SS, Sayyed, R. Z. (2015) Role of plant growth-promoting rhizobacteria and their formulation in biocontrol of plant diseases. In.: Maheshwari DK (Eds.) **Plant Microbes Symbiosis: Applied Facets**. New Delhi: Springer, p: 337–351.

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. **SpringerPlus** 2:1-14.

Shaterian J, Waterer D, De Jong H, Tanino KK (2005) Differential stress responses to NaCl salt application in early- and late-maturing diploid potato (*Solanum* sp.) clones. **Environmental and Experimental Botany** 54: 202–212.

Sheng XF, Zhao F, He LY, Qiu G, Chen L (2008) Isolation and characterization of silicate mineral-solubilizing *Bacillus globisporus* Q12 from the surfaces of weathered feldspar. **Canadian Journal of Microbiology** 54: 1064–1068.

Sherathia D, Dey R, Thomas M, Dalsania T, Savsani K, Pal KK (2016) Biochemical and molecular characterization of DAPG-producing plant growth promoting rhizobacteria (PGPR) of groundnut (*Arachis hypogaea* L.). **Legume Research** 39: 614–622.

Shiferaw B, Prasanna BM, Hellin J, Banziger M (2011) Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. **Food Security** 3:307–327.

Sitbon F, Hennion S, Sundberg B, Little CHA, Olsson O, Sandberg G (1992) Transgenic tobacco plants coexpressing the *Agrobacterium-tumefaciens-iaaM* and *iaaH* genes display altered growth and indoleacetic-acid metabolism. **Plant Physiology** 99: 1062–1069.

Solano BR, Maicas JB, Mañero FG (2008) Physiological and molecular mechanisms of plant growth promoting rhizobacteria (PGPR). In.: Ahmad I, Pichtel J, Hayat S (Eds.) **Plant-Bacteria Interactions: Strategies and Techniques to Promote Plant Growth**. Weinheim: Wiley p: 41–52.

Soutar CD, Stavrinides J (2018) The evolution of three siderophore biosynthetic clusters in environmental and host-associating strains of *Pantoea.* **Molecular Genetics and Genomics** 293: 1453–1467.

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions**. Cold Spring Harbor Perspectives in Biology** 3: 1–13.

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. **FEMS Microbiology Reviews**. 31: 425–448.

Sparks DL, Huang PM (1985) Physical chemistry of soil potassium. In.: Munson **Potassium in Agriculture**, p. 201–276.

Spolaor LT, Gonçalves LSA, Santos OJAPD, Oliveira ALMD, Scapim CA, Bertagna FAB, Kuki MC (2016) Plant growth-promoting bacteria associated with nitrogen fertilization at topdressing in popcorn agronomic performance. **Bragantia** 75: 33–40.

Syed S, Chinthala P (2015) Heavy metal detoxification by different *Bacillus species* isolated from solar salterns. **Scientifica** 2015: 1-8.

Tabassum B, Khan A, Tariq M, Ramzan M, Khan MSI, Shahid N, Aaliyaa K (2017) Bottlenecks in commercialisation and future prospects of PGPR. **Applied Soil Ecology** 121: 102–117.

Tahir HAS, Gu Q, Wu HJ, Raza W, Hanif A, Wu LM, Colman MV, Gao X (2017). Plant growth promotion by volatile organic compounds produced by *Bacillus subtilis* SYST2. **Frontiers in Microbiology** 8: 1-11.

Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moenne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Prigent-Combaret C (2013) Plant growth-promoting rhizobacteria and root system functioning. **Frontiers in Plant Science** 4: 1-19.

Van Loon LC (1997) Induced resistance in plants and the role of pathogenesis-related proteins. **European Journal of Plant Pathology** 103: 753–765.

Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. **European Journal of Plant Pathology** 119: 243–254.

Venturi V, Keel C (2016) Signaling in the rhizosphere. **Trends Plant Sci** 21:187-198.

Wang GL, Que F, Xu ZS, Wang F, Xiong AS (2015) Exogenous gibberellin altered morphology, anatomic and transcriptional regulatory networks of hormones in carrot root and shoot. **BMC Plant Biology** 15: 1-12.

Wang N, Hua HB, Eneji AE, Li ZH, Duan LS, Tian XL (2012) Genotypic variations in photosynthetic and physiological adjustment to potassium deficiency in cotton (*Gossypium hirsutum*). **Photochemistry and Photobiology B: Biology** 110: 1–8.

Wani PA, Khan MS (2014) Screening of multiple metal and antibiotic resistant isolates and their plant growth promoting activity. **Pakistan Journal of Biological Sciences** 17: 206–212.

Woodward AW, Bartel B (2005) Auxin: Regulation, action, and interaction. **Annals of Botany** 95: 707–735.

Yu H, Ling N, Wang T, Zhu C, Wang Y, Wang S, Gao Q (2019) Responses of soil biological traits and bacterial communities to nitrogen fertilization mediate maize yields across three soil types. **Soil & Tillage Research** 185: 61–69.

Zeevaart J (1999) Abscisic acid metabolism and its regulation. In.: Hooykaas P, Hall M, Libbeng K (Eds.) **Biochemistry and Molecular Biology of Plant Hormones**. Amsterdam: Elsevier, p. 189–207.

Zhang WL, Zhang Y, Wang XX, Ding FS, Fu YM, Zhao JZ, Song W, Opiyo OJ, Zhang F, Chen X (2017) Siderophores in clinical isolates of *Klebsiella pneumoniae* promote ciprofloxacin resistance by inhibiting the oxidative stress. **Biochemical and Biophysical Research Communications** 491: 855–861.

Zhao DL, Li YR (2015) Climate change and sugarcane production: potential impact and mitigation strategies. **International Journal of Agronomy** 2015: 1–10.

Zhao DL, Oosterhuis DM, Bednarz CW (2001) Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. **Photosynthetica** 39: 103–109.

CAPÍTULO 2 - Selection of *Saccharum* spp. rhizobacteria with growth-promoting properties using PCA analysis[1]

Abstract - The search for plant growth-promoting rhizobacteria is an ongoing need for the development of new bioinoculants for use in various crops, including sugarcane. Bacterial strains with various plant growth-promoting properties can contribute to sustainable agricultural production. The present study aimed to isolate, characterize and select sugarcane rhizobacteria from six different varieties through principal components analysis. This study selected 167 bacterial strains with the ability to fix nitrogen, produce indolacetic acid, exhibit cellulolytic activity, and solubilize phosphate and potassium were isolated. Of these 167 bacterial strains, seven were selected by principal component analysis and identified as belonging to the genera *Staphylococcus*, *Enterobacter*, *Bacillus* and *Achromobacter*. *Bacillus thuringiensis* IP21 presented higher potential for nitrogen fixation and $CaPO_4$ and $AlPO_4$ solubilization and a lower potential for K solubilization. *Enterobacter asburiae* IP24 was efficient in indolacetic acid production and $CaPO_4$ and $FePO_4$ solubilization and inefficient for Araxá apatite solubilization. The bacterial strains isolated in the present study showed great variations in terms of the expression of their abilities to promote plant growth, and more studies are needed to confirm whether these strains can perform plant growth.

Keywords: Plant production; plant growth promoting rhizobacteria; sugarcane.

Abbreviations: PGPR: plant growth-promoting rhizobacteria; PCA: principal component analysis; BNF: biological nitrogen fixation; IAA: indolacetic acid.

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Introduction

Although the industrialization of agriculture has brought about major changes in the agricultural production system, with significant increases in productivity, it has also caused serious environmental problems that must be addressed and resolved in the near future. Currently, a major challenge faced by professionals working in the agricultural sector is maintaining productivity with reduced production costs and lower environmental impact. In this context, an alternative is the use of plant growth-promoting rhizobacteria (PGPR) (Pérez-Montaño et al., 2014). This term is used to describe a specific category of microorganisms involved in some root-microorganisms-plant interactions that can bring several benefits to plants by promoting plant growth (Zhou et al., 2016).

Plant growth-promoting rhizobacteria (PGPR) are widely used in crop production and appeared to be an environmental-friendly approach for improvement in the growth of the plant and soil fertility (Chaturvedi et al., 2020). PGPR produce several compounds, including growth regulation (phytohormones), such as the synthesis of auxins, cytokines, gibberellins, ethylene, and abscisic acid, which help plants in their growth process, such as root extension and cell division. They are also capable of producing siderophores and organic acids, fixing atmospheric nitrogen (N_2), solubilizing phosphate and potassium. Therefore, increase in nutrient use efficiency, solubilization of insoluble phosphates and potassium, and chelation of micronutrients (Di Salvo et al., 2018; Hayat et al., 2012; Rana et al., 2012; Roesti et al., 2006; Sheng and He, 2006) which may influence the native soil microbial community due to differential plant growth promotion action. Addition the PGPR can produce antibiotics to suppress pathogenic rhizobacteria. These substances directly and indirectly affect plant metabolism and improve the adaptive capacity of plants to absorb soil nutrients (Grobelak et al., 2015).

Microbial community structure and enzyme activities in the soil are indices for assessment of soil health and quality which are responsible for biogeochemical processes and nutrients transformation (Di Salvo et al., 2018; Hayat et al., 2012). PGPR require to establish and sustain a critical bacterial population in the soil for effective plant growth enhancement and interact with indigenous soil microbes (Kang

et al., 2013); therefore, soil microbial ecological studies are necessary (Di Salvo et al., 2018). For commercial exploitation of PGPR as an efficient biofertilizer, it is essential to examine the interaction of native soil microbial community structure and functions for potential ecological impacts. The ecological impact is an important and essential aspect for the influence of PGPR on microbial community study for safe and consistent utilization of PGPR at large scale. The soil inoculation of large extent of exogenous bacteria as PGPR has the probable impact on the indigenous microbes and inoculant may affect them which may results in increase, decrease or no influence on native microbes activities (Bharti et al., 2015; Domenech et al., 2004; Garcıá et al., 2004; Li et al., 2018) which warrants the need to be study the soil microbial ecology using new analytical and molecular tools.

Although some PGPR promote plant growth, some rhizobacteria-based inoculants may have little or no growth-promoting effect on sugarcane crop, and the selection of new bacterial strains isolated from the own crop with diverse growth-promoting properties may be necessary. With this process of bacterial strain selection, since many strains have diverse plant growth-promoting properties, it is difficult to select the most appropriate bacterial strain as a future bioinoculant (Li et al., 2018)

Given the above, the present study aimed to isolate, characterize and select rhizobacteria in six different varieties of sugarcane carriers of abilities to promote plant growth that could be used as inoculant.

Results

Screening of bacteria carriers of plant growth abilities

Sixty bacterial strains were isolated from the rhizospheres of the IAC95-5000 and RB86-7515 varieties in the municipality of Jaboticabal-SP; 62 colonies from the CTC9 and RB85-5156 varieties in the municipality of Frutal-MG and 45 colonies from the IAC91-1099 and CTC4 varieties in the municipality of Pirajuba-MG. A total of 167

bacterial colonies were isolated from the three locations, of which 58 had the capacity to fix N, 20 had the capacity to produce IAA, 53 had cellulolytic activity, and 17, 26, 44, 33, 51 had the capacity to solubilize K, $CaPO_4$, $AlPO_4$, $FePO_4$ and Araxá apatite, respectively (Table 1).

Table 1. Total number of bacteria strains with BNF, IAA production, cellulolytic activity (CA), K, $CaPO_4$, $AlPO_4$, $FePO_4$, and Araxá apatite solubilization properties isolated from the IAC95-5000, RB86-7515, CTC9, RB75-5156, IAC91-1099, CTC4 varieties in the municipalities of Jaboticabal, Frutal and Pirajuba.

Municipality	Jaboticabal		Frutal		Pirajuba	
Variety	IAC95-5000	RB86-7515	CTC 9	RB75-5156	IAC91-1099	CTC4
Strain code	IJ	RJ	CF	RF	IP	CP
Total strains	33	27	29	33	24	21
BNF	7	15	6	9	12	8
IAA	1	7	1	0	7	4
CA	7	14	5	6	12	8
Solubilization:						
K	2	3	0	0	8	3
$CaPO_4$	5	7	2	4	4	3
$AlPO_4$	2	11	4	5	12	8
$FePO_4$	3	7	3	3	11	5
Apatite	7	11	6	6	12	8

The joining of the two principal components allowed a two-dimensional ordering of isolates and variables, thus enabling the construction of a biplot graph (Fig. 1). The amount of total information of original variables retained by the two principal components was 50.79%, 32.36% in the first principal component and 18.43% in the second principal component (Fig. 1).

The order of isolates according to the first two main components allocated isolates into two large groups. The graphical representation allowed for the determination of the variables that most discriminate in the formation of groups I and II. Variables IAA, K, $AlPO_4$, $FePO_4$ and Araxá apatite solubilization are responsible for the discrimination of group I, located on the left of PC1, exerting great influence on these isolates. Cellulolytic activity and $CaPO_4$ exerted weak influences on group I. Group II did not present any variable with a strong influence on itself; all characteristics showed no effect. Thus, group I was characterized by isolates with the characteristics

of being IAA producers and K, $AlPO_4$, $FePO_4$ and Araxá apatite solubilizers; therefore, group I was the group of interest and was selected for the next experiments (Fig. 1).

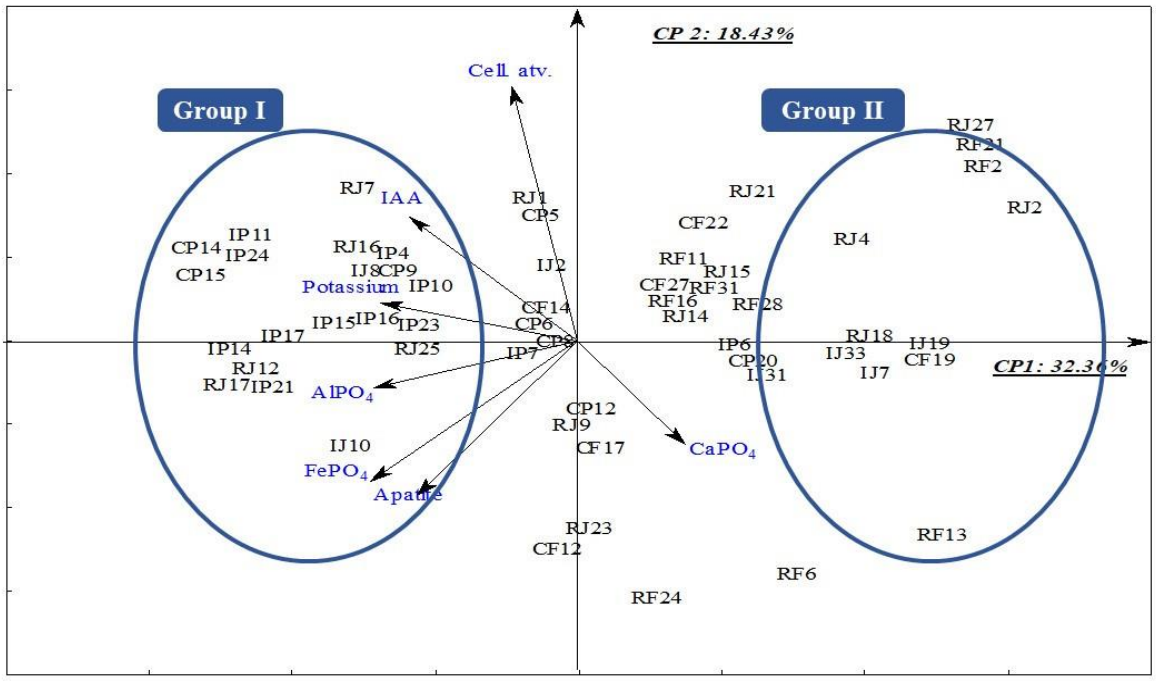

Fig 1. Dispersion (biplot graph) of qualitatively analyzed growth-promoting characteristics, namely cellulolytic activity (Cell.act), IAA production (IAA), K, $AlPO_4$, $FePO_4$, $CaPO_4$ and Araxá apatite solubilization.
*Strain code: First letter: initial of the variety used for isolation (IAC95-5000, RB86-7515, CTC9, RB75-5156, IAC91-1099 and CTC4). Second letter: initial of the municipality where the isolation was performed (Jaboticabal, Frutal and Pirajuba). Numeric code: number assigned to the strain according to the sequence in which it was isolated.

In the municipality of Jaboticabal, 22 bacterial strains were selected after BNF and only 07 were allocated in group I. In the municipality of Pirajuba, 13 of the 20 isolates selected after BNF were allocated in group I. For strains isolated in the municipality of Frutal, no strains were allocated in group I, so these strains were not considered for quantitative characterization. Quantitative analyses were performed on 20 bacterial strains selected after principal component analysis (PCA).

Screening the bacteria which presented the better results

The first two principal components presented 61.85% of the total data variance, with PC1 showing 42.39% of variance and PC2, 19.56%. The variables presented were responsible for the formation of three groups of interest. The variables $AlPO_4$, $FePO_4$ solubilization, IAA production and cellulolytic activity exerted a strong influence on group I, where isolates IP11 (*Enterobacter* sp.), IP14 and IP24 (*Enterobacter asburiae*) were grouped and responsible for their discrimination. BNF, $CaPO_4$ and Araxá apatite solubilization were responsible for the distinction of group II, which included isolates IJ8 (*Staphylococcus saprophyticus*), IP17 (*Bacillus anthracis*) and IP21 (*Bacillus thuringiensis*). Group III was influenced by only one variable, K solubilization, and the isolate IP23 (*Achromobacter spanius*) was grouped in group III (Fig. 2).

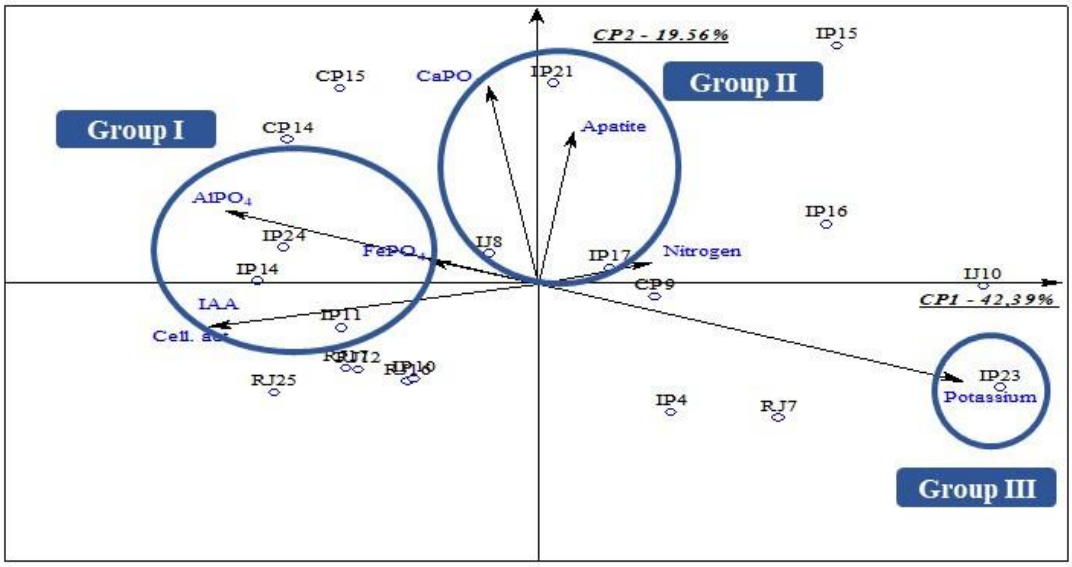

Fig 2. Dispersion (biplot graph) of the quantitatively analyzed growth-promoting characteristics, namely, BNF quantification, cellulolytic activity (Cell.Act), IAA production, K, $AlPO_4$, $FePO_4$, $CaPO_4$ and Araxá apatite solubilization.

Of isolates selected by PCA, six were from Pirajuba (IP) and only one was from Jaboticabal (IJ) (Fig. 2). For isolates that did not group, their molecular identification was not performed. Therefore, molecular identification was performed on seven bacterial strains.

For IAA production, the largest producers were IP14 and IP24 (*E. asburiae*), with 56.68 and 56.21 µg of IAA mL^{-1}, respectively. For cellulolytic activity and K

solubilization, isolate IP23 (*A. spanius*) stood out, producing 0.61 U mL^{-1} and 17.23 mg K mL^{-1}, respectively. For BNF, isolates IP21 (*B. thuringiensis*) and IJ8 (*S. saprophyticus*) were the major fixators, with 108.07 and 105.61 µg N mL^{-1}, respectively (Fig. 3).

For $CaPO_4$ and $AlPO_4$ solubilization, isolate IP21 (*B. thuringiensis*) stood out, solubilizing 481.00 and 39.33 mg of P mL^{-1}, while isolate IP17 (*B. anthracis*) was the best for Araxá apatite solubilization and IP14 stood out in $FePO_4$ solubilization, with 622.99 and 105.66 mg of P mL^{-1}, respectively (Fig. 3).

Molecular identification of bacterial strains

The selected strains that formed the three main groups according to PCA were identified using PCR amplification of the 16S rRNA gene. All isolates were identified by the 16S rDNA sequence and showed more than 95% homology with other sequences deposited at GenBank (Table 2).

Table 2. Identification of sugarcane rhizospheric bacteria using NCBI BLAST-N of 16S rRNA gene sequences.

Isolate	Species identification	Identity (%)
IJ8	*Staphylococcus saprophyticus* NR_114090.1	99.76
	Staphylococcus saprophyticus MG694483.1	100.00
IP11	*Enterobacter* sp. KR558701.1	99.85
	Enterobacter sp. HM748078.1	99.85
IP14	*Enterobacter* sp. KR558701.1	96.34
	Enterobacter sp. HM748078.1	96.34
IP17	*Bacillus anthracis* MK575034.1	99.88
	Bacillus anthracis AF290553.	99.88
IP21	*Bacillus thuringiensis* NR_112780.1	99.79
	Bacillus thuringiensis KT159186.1	99.68
IP23	*Achromobacter spanius* MN007235.1	99.29
	Achromobacter spanius NR_025686.1	99.29
IP24	*Enterobacter asburiae* MG571735.1	99.84
	Enterobacter asburiae KY316493.1	99.84

* Query cov., 100%; and E-value, 0.0, for all sequence

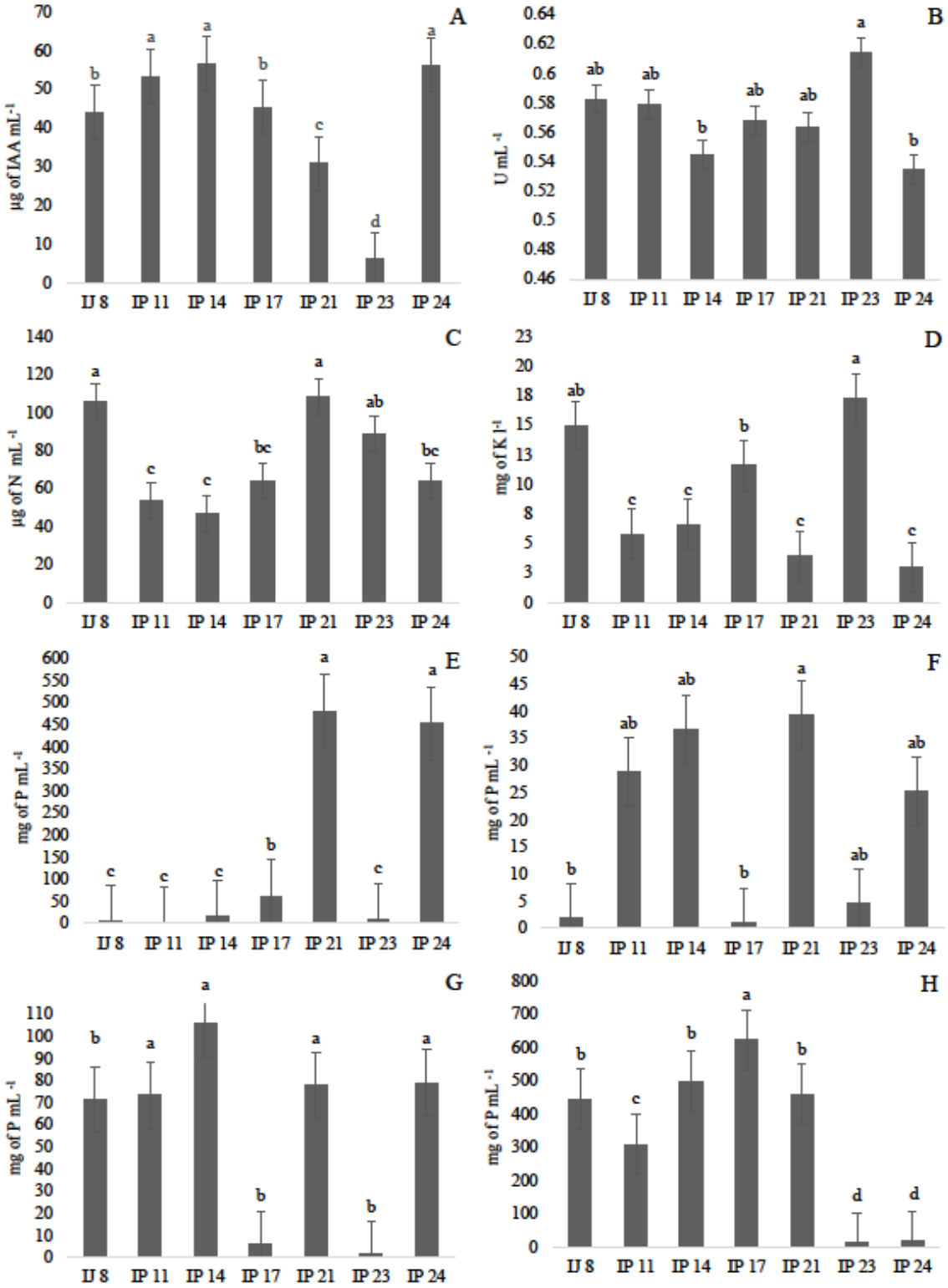

Fig 3. Quantitative results for a) IAA production; b) cellulolytic activity; c) BNF; d) K solubilization; e) $CaPO_4$ solubilization; f) $ALPO_4$ solubilization; g) $FePO_4$ solubilization and; h) Araxá apatite solubilization. Means followed by the same letters do not differ according to the Tukey test at 5% probability.
*_S. saprophyticus_ IJ8, _Enterobacter_ sp. IP11, _B. anthracis_ IP17, _B. thuringiensis_ IP21, _A. spanius_ IP23, _E. asburiae_ IP24.

Isolate IJ8 was homologous to sequences of *Staphylococcus saprophyticus* and showed more than 99% identity with two sequences. The identification of isolate IP11 was performed at the genus level, demonstrating 99.85% homology with two *Enterobacter* sequences. Isolate IP14 showed over 95% homology with the genus *Enterobacter*. Isolate IP17 was identified as *Bacillus anthracis* based on 99.88% sequence identity. The identification of isolate IP21 was performed according to homology with sequences of *Bacillus thuringiensis*, with identity above 99.5%. Isolate IP23 was identified from homology with sequences of *Achromobacter spanius*, with 99.29% sequence homology. Finally, isolate IP24 was homologous with *Enterobacter asburiae* sequences with 99.84% identity (Table 2).

A phylogenetic tree was constructed to demonstrate similarity between bacterial strain sequences of the present study and sequences deposited in GenBank, in addition to demonstrating similarity to each other (Fig. 4).

Discussion

The present study used PCA to determine the statistical correlation between isolates and growth promotion characteristics. Figure 1 shows that group I was characterized by isolates that produce IAA and solubilize K, $AlPO_4$, $FePO_4$, and Araxá apatite. Group II has no growth-promoting variables of interest (Fig. 1).

Sugarcane is a plant, and a large variety of bacteria have been isolated, characterized and identified in its rhizosphere. Through PCA, the present work was able to isolate for the first time *S. saprophyticus, E. asburiae, A. spanius* and *B. anthracis* strains in the sugarcane rhizosphere. In addition, of the seven strains identified, there was no repetition of species; therefore, the strains were seven distinct species.

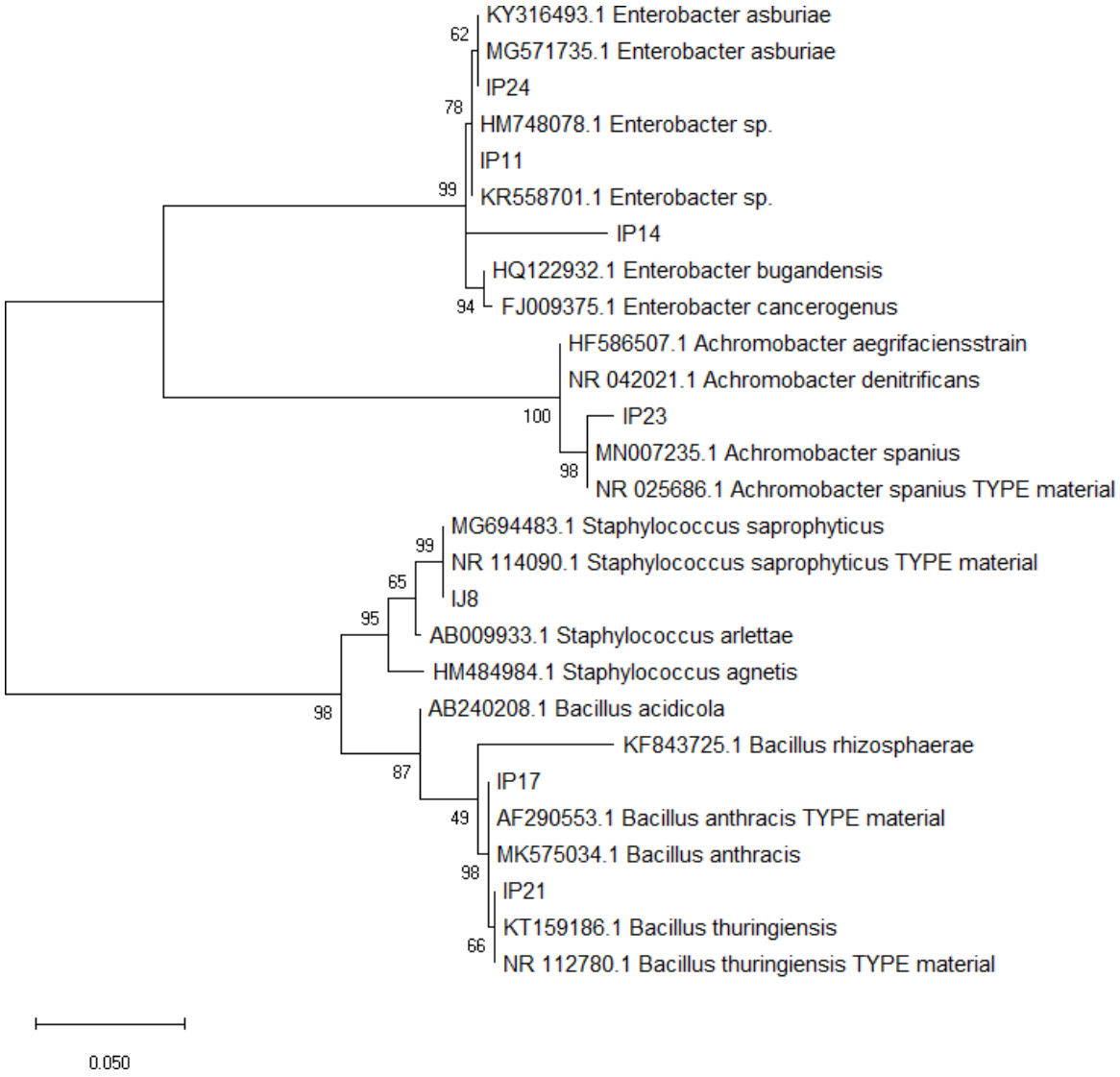

Fig 4. Phylogenetic analysis of the 16S ribosomal region of the seven strains isolated and grouped into the three groups by PCA (Fig. 2). The phylogenetic tree was constructed based on the maximum probability with the evolutionary method and distances calculated according to the Kimura-2 parameter. Boot values are presented as a percentage for 1000 bootstraps. Numeric codes before species names refer to the GenBank (NCBI) accession number.

The *S. saprophyticus* IJ8 strain stood out for BNF (105.61 µg of N mL^{-1}) and $CaPO_4$ (4.67 mg P mL^{-1}) and Araxá apatite solubilization (444.00 mg P mL^{-1}) (Fig. 3), important characteristics to be considered for PGPR. Strains that exhibit efficient N fixation and P solubilization tend to be good inoculants because N-fixing PGPR inoculation in crops revitalizes plant growth, promoting activities, the management of diseases and low nitrogen levels in agricultural soils (Damam et al., 2016). Phosphorus

is the second most essential nutrient demanded by plants, and in adequate amounts for optimal growth, it plays an important role in almost all major metabolic processes, including energy transfer, signal transduction, respiration, macromolecular biosynthesis and photosynthesis (Anand et al., 2016).

Members of the genus *Staphylococcus* are well known human pathogens, but some species have been isolated from soil (Zamil et al., 2010), wild rice (Sarathambal et al., 2015), grape (Barata et al., 2012), and oil-contaminated samples (Silva et al., 2015), suggesting their opportunistic mode of existence and demonstrating wide variation in the environment where they can be found. Some authors searching for new growth-promoting isolates have been able to characterize and identify *S. saprophyticus* isolates.

Isolates IP11 and IP14 had high similarity with sequences of *Enterobacter* sp. (Table 2), but when the phylogenetic tree was constructed, IP14 did not group with GenBank database sequences, indicating that this isolate could represent some species not yet identified (Fig. 4). Elo et al. (2000) analyzed 16S rDNA sequences from bacteria isolated from the humus layer and found unknown *Paenibacillus* species. Similarly, Beneduzi et al. (2013) found rhizobacteria isolated from sugarcane, and some isolates had no similarity to any sequence in the available databases.

Enterobacter sp. is a bacterium that has been found and classified only at the genus level by several researchers searching for PGPR (Sajjad Mirza et al., 2001). Waghmare et al. (2018) demonstrated the potential of *Enterobacter* sp. as a cellulolytic bacterium. Patel et al. (2019) found a phosphorus-solubilizing and IAA-producing strain, as well as in the present study, where *Enterobacter* sp. IP11 produced 53.32 µg of IAA mL^{-1} (Fig. 3). *Enterobacter* sp. has also been isolated from chromium-contaminated soils suggesting that it is resistant to chromium and promoting the growth of tomato plants under Cr stress conditions (Gupta et al., 2019).

E. asburiae is also known to be an opportunistic pathogen, but its strains have been isolated from a wide variety of environments, including rhizosphere and agricultural soil (Gyaneshwar et al., 1999), cotton (Quadt-Hallmann and Kloepper, 1996), *Arabidopsis thaliana* (Cooley et al., 2003), mustard (Ahemad and Kibret, 2014), tobacco (Ahemad and Khan, 2010; Zhang and Kong, 2014), and sugarcane roots (Kruasuwan and Thamchaipenet, 2016).

E. asburiae IP24 could be used as a growth promoter, standing out in $FePO_4$, $CaPO_4$ solubilization (79.00 and 452.67 mg P mL^{-1}, respectively) and IAA production (56.21 µg IAA mL^{-1}) (Fig. 3). In the search for growth-promoting sugarcane endophytes, Kruasuwan and Thamchaipenet (2016) isolated an *E. asburiae* strain, but this strain was negative for P solubilization, Ahemad and Khan (2010) when characterizing *E. asburiae*, they also observed their growth-promoting characteristics; the strain produced 32 µg of IAA mL^{-1}, while the strain of this study produced 56.21 µg of IAA mL^{-1}, almost twice the value obtained by the authors. Because nutritional elements are absorbed from soil by roots, good root growth is considered a prerequisite for increased plant growth, and IAA is one of the most important plant hormones produced by bacteria, improving the protection level against adverse external effects and increasing the coordination of various cell defense systems (Spaepen and Vanderleyden, 2011).

A. spanius IP23 stood out for K solubilization (17.3 mg K^{-1}) and cellulolytic activity (0.61 U mL^{-1}) (Fig. 3). Previously, *A. spanius* was found in medicinal plants, being positive for cellulolytic activity, but with low IAA production (4.2 µg IAA mL^{-1}) (Egamberdieva et al., 2017); this result was similar to the present study, where the *A. spanius* isolate produced the lowest IAA concentration (6.30 µg of IAA mL^{-1}). *A. spanius* has also been found in soil (Castanheira et al., 2014) and characterized for growth promotion in *Phragmites australis* (Soares et al., 2016) and ryegrass (*Lolium multiflorum*) (Castanheira et al., 2014).

When constructing the phylogenetic tree, isolate IP21 had a low bootstrap value (Fig.4), which may be explained by the genetic similarity of *B. anthracis* and *B. thuringiensis*. These species are very close to each other, and some studies suggest that *B. anthracis* is a narrow group of strains that show a high degree of genetic similarity and that inclusions of bioinsecticide crystals are the only distinguishing feature of *B. thuringiensis* (Jensen et al., 2003). Sometimes, phylogenetic analysis based on the 16S rRNA gene sequence is not able to differentiate *B. anthracis* and *B. thuringiensis* (Jamil, 2015; Tchuisseu Tchakounté et al., 2018).

Despite being known as an animal and human pathogen, *B. anthracis* strains were previously found in the search for tomato rhizosphere growth promoters, where they were positive for N fixation and IAA production (± 6.45 µg IAA mL^{-1}) (Tian et al.,

2017). The *B. anthracis* IP17 strain of the present study produced 45.34 µg IAA mL^{-1} and stood out for Araxá apatite solubilization, 623.00 mg P mL^{-1} (Fig. 3). Other authors have managed to isolate *B. anthracis* from *Phragmites australis* (L.) rhizosphere (Chaturvedi et al., 2006) and atrazine-contaminated soils (El-Bestawy et al., 2013), but these authors did not observe whether these strains presented growth-promoting properties. No reports of *B. anthracis* associated with sugarcane were found in the literature regarding rhizospheric or endophytic bacteria. The isolate *B. anthracis* IP17 could be cited as the first report found in the sugarcane rhizosphere.

B. thuringiensis is well known for its specific bioactivity, due to its capacity to produce crystals, which are formed by polypeptides known as Cry proteins. These proteins have entomopathogenic properties for insects of the orders Lepidoptera, Diptera, Coleoptera, Hymenoptera, and Homoptera, in addition to nematodes, protozoa and mites (Jensen et al., 2003). In sugarcane, *B. thuringiensis* has been intensively studied for the control of borer (*Diatraea saccharalis*) (Huang et al., 2008; Wu et al., 2009), but there are few studies that directly relate this species as a growth promoter in sugarcane and other crops.

B. thuringiensis strains that were positive for P solubilization and IAA production were isolated from *Kobresia capillifolia* (de Freitas et al., 1997; Ying et al., 2016). The *B. thuringiensis* IP21 strain was a great solubilizer of the phosphate sources tested (481.00, 39.33, 77.66, 457.66 mg P mL^{-1} for $CaPO_4$, $FePO_4$, $AlPO_4$ and Araxá apatite, respectively), in addition to being the strain that fixed the largest amount of N, 108.07 µg N mL^{-1}. *B. thuringiensis* has also been isolated from *Pinus Sylvestris* (Babu et al., 2013).

Materials and methods

Rhizospheric soil

Sugarcane rhizospheric soil samples were collected in the municipality of Jaboticabal - SP (21º 15 '17 "S and 48º 19' 20" W) and included the IAC95-5000 and RB86-7515 varieties. Collection was also performed in the municipality of Pirajuba - MG (19 ° 54 '32" S and 48 ° 42' 9 'W), the IAC91-1099 and CTC4 varieties, and in the municipality of Frutal - MG (20º 01' 29 " S and 48º 56 '26 "W), the CTC9 and RB85-5156 varieties. Soil samples were collected and transported to the Laboratory of Agricultural Microbiology, UNESP, Campus of Jaboticabal. Field permits were not required for this research. In Jaboticabal, the land we sampled belongs to our institution (State University of São Paulo, UNESP).

Bacteria from rhizospheric soil samples were isolated by serial dilution (Wollum, 1982; Vieira and Nahas, 2005). After incubation, bacterial colonies were picked, placed into SMA-containing test tubes and refrigerated for later use.

Screening of isolates

All strains were tested for biological nitrogen fixation capacity (BNF) in plates, and strains with this characteristic were then tested for phosphate solubilization ($CaPO_4$, $AlPO_4$, $FePO_4$ and Araxá apatite), potassium solubilization (Ekosil®), indolacetic acid (IAA) production and cellulolytic activity.

Through PCA, bacterial strains that presented the best results for the parameters mentioned above were selected. For the quantification of plant growth promotion properties, all strains were grown for 24 h in nutrient broth at a concentration of 1×10^8 colony forming units (CFU). Analyses were performed in triplicate, and media without the presence of bacteria were used as controls.

Biological Nitrogen Fixation (BNF)

Nitrogen fixation capacity was evaluated according to Dobereiner et al. (1996) and Tedesco et al. (1995).

Phosphate solubilization

Phosphate solubilization based on bacterial culture was measured according to Berraquero et al. (1976). Four phosphate sources were tested: $CaPO_4$, Araxá apatite ($3Ca_3(PO_4)2CaF_2$), $AlPO_4$, and $FePO_4$ in the amounts of 5 g, 5 g, 3.5 g, and 4.33 g, respectively, per 1 L of medium (Silva Filho and Vidor, 2000).

Potassium Solubilization

Potassium solubilization was measured using Ekosil® fertilizer, which is an alternative source of K produced from rock of volcanic origin called phonolite, containing 8% soluble K_2O (Yoorin, 2018).

IAA production

IAA production was measured according to Sarwar and Kremer (1995).

Cellulolytic activity

The cellulolytic activity was measured by Ramachandra et al. (1987) and Ghose (1987).

Data analysis

Data were initially submitted to the PCA multivariate statistical method to group bacterial strains, which was performed after standardization of variables in which each variable had a mean of 0 and a variance of 1. A biplot was constructed for the first two principal components with isolates and growing promotion characteristics. STATISTICA software version 7.0 was used for processing statistical analyses (Statsoft, 2014).

Quantitative data were also subjected to analysis of variance (F-test), comparing treatment means by the Tukey test at 5% probability using AgroEstat software version 1.0 (Barbosa and Maldonado, 2010).

Molecular identification of bacterial strains

Strains that showed the best results consistent with previously described properties were identified following the Quick-DNA Universal extraction kit protocol (ZymoResearch - cat, No. D4068 and D4069) (Sambrook and Fritsch, 1989).

For identification, PCR products were purified using the Wizard® SV Gel and PCR Clean-up System Kit and sequenced using universal primers. Sequences were edited using the Biological Sequence Alignment Editor – BioEdit (Hall, 1999); the consensus sequence was obtained using the BLAST® tool (Altschul et al., 1990) and compared with the National Center for Biotechnology Information (NCBI - GenBank) database. The resulting phylogenetic trees were constructed using MEGA7® software (Tamura et al., 2011).

Conclusion

The PCA was a great strategy and a useful tool to select rhizobacteria strains from sugarcane, such as *S. saprophyticus, E. asburiae, A. spanius* e *B. anthracis.* These bacteria showed similar behavior and expressed important characteristics related to plant growth-promoting. However, more studies are needed to verify if these selected strains will show positive aspects on sugarcane plants, including an increase of yield.

References

Ahemad M, Khan M S (2010) Plant growth promoting activities of phosphate-solubilizing *Enterobacter asburiae* as influenced by fungicides. Eurasia J Biosci. 88–95.

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. JKSUES. 26:1–20.

Altschul SF, Gish W, Miller W, Myers EW Lipman DJ (1990) Basic local alignment search tool. J. Mol. Biol 3:403-410.

Anand K, Kumari B, Mallick MA (2016) Phosphate solubilizing microbes: an effective and alternative approach as biofertilizers. J Pharm Pharm Sci. 8:37-4.

Babu AG, Kim JD, Oh BT (2013) Enhancement of heavy metal phytoremediation by *Alnus firma* with endophytic *Bacillus thuringiensis* GDB-1. J Hazard Mater. 250:477-483.

Barata A, Malfeito-Ferreira M, Loureiro V (2012) Changes in sour rotten grape berry microbiota during ripening and wine fermentation. Int J Food Microbiol. 3:152–161.

Barbosa JC, Maldonado JW (2010) AgroEstat: sistema para análises estatísticas de ensaios agronômicos. Versão 1.0.

Beneduzi A, Moreira F, Costa PB, Vargas LK, Lisboa BB, Favreto R, Baldani JI, Passaglia, L MP (2013) Diversity and plant growth promoting evaluation abilities of bacteria isolated from sugarcane cultivated in the South of Brazil. Appl Soil Eco. 63:94–104.

Berraquero FR, Baya B, Cormenzana AR (1976) Establecimiento de índices para el estudio de la solubilización de fosfatos por bacterias del suelo. Ciencia del suelo. 17:399-406.

Bharti N, Barnawal D, Maji D & Kalra A (2015) Halotolerant PGPRs prevent major shifts in indigenous microbial community structure under salinity stress. Microb Ecol. 70(1):196-208.

Castanheira N, Dourado AC, Alves PI, Cortés-Pallero AM, Delgado-Rodríguez AI, Prazeres Â, Borges N, Sánchez C, Barreto Crespo MT, Fareleira P (2014) Annual ryegrass-associated bacteria with potential for plant growth promotion. Microbiol Res. 169:768–779.

Cavalcante VA, Dobereiner J (1988) A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. Plant Soil. 108:23–31.

Chaturvedi S, Chandra R, Rai V (2006) Isolation and characterization of P*hragmites australis* (L.) rhizosphere bacteria from contaminated site for bioremediation of colored distillery effluent. Ecol Eng. 27:202–207.

Chaudhary DR, Rathore AP & Sharma S (2020) Effect of halotolerant plant growth promoting rhizobacteria inoculation on soil microbial community structure and nutrients. Appl Soil Ecol 150:103461.

Cooley MB, Miller WG, Mandrell RE (2003) Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. Appl Environ Microbiol. 69:4915–4926.

Damam M, Kaloori K, Gaddam B, Kausar R (2016) Plant growth promoting substances (phytohormones) produced by rhizobacterial strains isolated from the rhizosphere of medicinal plants. Int J Pharm Sci Rev Res. 37:130-136.

Di Salvo LP, Cellucci GC, Carlino ME & de Salamone IEG (2018) Plant growth-promoting rhizobacteria inoculation and nitrogen fertilization increase maize (*Zea mays* L.) grain yield and modified rhizosphere microbial communities. Appl Soil Ecol. 126:113-120.

Domenech J, Ramos-Solano B, Probanza A, Lucas-García JA, Colón JJ & Gutiérrez-Mañero FJ (2004) *Bacillus* spp. and *Pisolithus tinctorius* effects on *Quercus ilex* ssp. ballota: a study on tree growth, rhizosphere community structure and mycorrhizal infection. Forest Ecol Manag 194(1-3):293-303.

De Freitas JR, Banerjee MR, Germida JJ (1997) Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). Biol Fertil Soils. 24:358–364.

Silva DDSP, de Lima Cavalcanti D, de Melo EJV, dos Santos PNF, da Luz ELP, de Gusmão NB, de Queiroz MDFV (2015) Bio-removal of diesel oil through a microbial consortium isolated from a polluted environment. Int Biodeter Biodegr. 97:85-89.

Dobereiner J, Baldani VLD, Baldani JI (1996) Como isolar e identificar bacterias diazotroficas de plantas nao-leguminosas. Embrapa-SPI.

Egamberdieva D, Wirth S, Behrendt U, Ahmad P, Berg G (2017) Antimicrobial activity of medicinal plants correlates with the proportion of antagonistic endophytes. Front Microbiol. 8:199.

El-Bestawy E, Sabir J, Mansy AH, Zabermawi N (2013) Isolation, identification and acclimatization of Atrazine-resistant soil bacteria. Ann Agric Sci. 58:119–130.

Elo S, Maunuksela L, Salkinoja-Salonen M, Smolander A, Haahtela K (2006) Humus bacteria of *Norway spruce* stands: plant growth promoting properties and birch, red fescue and alder colonizing capacity. FEMS Microbiol Ecol. 31:143–152.

García JAL, Domenech J, Santamaría C, Camacho Ma, Daza A & Mañero FJG (2004) Growth of forest plants (pine and holm-oak) inoculated with rhizobacteria: relationship with microbial community structure and biological activity of its rhizosphere. Environ Exp Bot. 52(3):239-251.

Malavolta E, Vitti GC, Oliveira SAD (1989) Avaliação do estado nutricional das plantas: princípios e aplicações.

Ghose TK (1987) Measurement of cellulase activities. Pure Appl Chem. 59:257–268.
Grobelak A, Napora A, Kacprzak M (2015) Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. Ecol Eng. 84:22–28.

Gupta N, Skinner KA, Khan S, Edirisinghe JN, Henry CS (2019) Draft genome sequence of *Enterobacter* sp. strain A8, a carbazole-degrading bacterium. Microbiol Resour Announc. 8:e00301-19

Gyaneshwar P, Parekh L, Archana G, Poole P, Collins M, Hutson R, Kumar GN (1999) Involvement of a phosphate starvation inducible glucose dehydrogenase in soil phosphate solubilization by *Enterobacter asburiae*. FEMS Microbiol Eco. 171:223–229.

Haidar B, Ferdous M, Fatema B, Ferdous AS, Islam MR, Khan H (2018) Population diversity of bacterial endophytes from jute (*Corchorus olitorius*) and evaluation of their potential role as bioinoculants. Microbiol Res. 208:43–53.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symp ser. 41:95–98

Hayat R, Ahmed I & Sheirdil RA (2012) An overview of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture. Crop production for agricultural improvement. Springer, p 557-579.

Hu X, Chen J, Guo J (2006) Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. World J Microbiol Biotechnol. 22:983–990.

Huang F, Leonard R, Moore S, Yue B, Parker R, Reagan T, Stout M, Cook D, Akbar W, Chilcutt C, White W, Lee D, Biles S (2008) Geographical susceptibility of Louisiana and Texas populations of the sugarcane borer, *Diatraea saccharalis* (F.) (*Lepidoptera: Crambidae*) to *Bacillus thuringiensis* Cry1Ab protein. Crop Prot 2:799–806.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual (Ed. 2). Cold spring harbor laboratory press.

Jamil M (2015) Plant Growth promoting rhizobacteria: An alternate way to improve yield and quality of wheat (*Triticum aestivum*). Proc Natl Acad Sci. 17:1.

Jensen GB, Hansen BM, Eilenberg J, cMahillon J (2003) The hidden lifestyles of *Bacillus cereu*s and relatives. Environ Microbiol. 5:631–640.

Kang Y, Shen M, Wang H & Zhao Q (2013) A possible mechanism of action of plant growth-promoting rhizobacteria (PGPR) strain Bacillus pumilus WP8 via regulation of soil bacterial community structure. J Gen Appl Microbiol. 59(4):267-277

Kruasuwan W, Thamchaipenet A (2016) Diversity of culturable plant growth-promoting bacterial endophytes associated with sugarcane roots and their effect of growth by co-inoculation of diazotrophs and actinomycetes. J Plant Growth Regul. 35:1074–1087.

Kuss AV, Kuss VV, Lovato T, Flôres ML (2007) Fixação de nitrogênio e produção de ácido indolacético *in vitro* por bactérias diazotróficas endofíticas. Pesq agropec bras. 42:1459–1465.

Li L, Ma J, Ibekwe AM, Wang Q & Yang C-H (2018) Influence of *Bacillus subtilis* B068150 on cucumber rhizosphere microbial composition as a plant protective agent. Plant and Soil 429(1-2):519-531.

Marcano IE, Díaz-Alcántara CA, Urbano B, González-Andrés F (2016) Assessment of bacterial populations associated with banana tree roots and development of successful plant probiotics for banana crop. Soil Biol Biochem. 99:1–20.

Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ (1995) Análises de solo, plantas e outros materiais 174:Porto Alegre: Ufrgs.

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Eco. 170:265–270.

Patel P, Shah R, Joshi B, Ramar K, Natarajan A (2019) Molecular identification and biocontrol activity of sugarcane rhizosphere bacteria against red rot pathogen *Colletotrichum falcatum.* Biotechnol Rep*.* 21:e00317.

Pérez-Montaño F, Alías-Villegas C, Bellogín RA, Del Cerro P, Espuny, MR, Jiménez-Guerrero I, López-Baena FJ, Ollero FJ, Cubo T (2014) Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. Microbiol Res. 169:325–336.

Quadt-Hallmann A, Kloepper JW (1996) Immunological detection and localization of the cotton endophyte *Enterobacter asburiae* JM22 in different plant species. Can J Microbiol. 42:1144–1154.

Ramachandra M, Crawford DL, Pometto AL (1987) Extracellular Enzyme activities during lignocellulose degradation by *Streptomyces* spp.: a comparative study of wild-type and genetically manipulated strains. Appl Environ Microbiol. 53:2754–2760.

Rana A, Saharan B, Joshi M, Prasanna R, Kumar K & Nain L (2011) Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. Ann Microbiol 61(4):893-900.

Roesti D, Gaur R, Johri B, Imfeld G, Sharma S, Kawaljeet K & Aragno M (2006) Plant growth stage, fertiliser management and bio-inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria affect the rhizobacterial community structure in rain-fed wheat fields. Soil Biol Biochem. 38(5):1111-1120.

Sajjad Mirza M, Ahmad W, Latif F, Haurat J, Bally R, Normand P, Malik KA (2001) Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane in vitro. Plant soil. 237:47–54.

Sarathambal C, Ilamurugu K, Balachandar D, Chinnadurai C, Gharde Y (2015) Characterization and crop production efficiency of diazotrophic isolates from the rhizosphere of semi-arid tropical grasses of India. Appl Soil Ecol. 87:1–10.

Sheng XF & He LY (2006) Solubilization of potassium-bearing minerals by a wild-type strain of Bacillus edaphicus and its mutants and increased potassium uptake by wheat. Can J Microbiol 52(1):66-72.

Sarwar M, Kremer RJ (1995) Determination of bacterially derived auxins using a microplate method. Lett Appl Microbio. 20: 282–285.

Silva Filho GN, Vidor C (2000) Solubilização de fostatos por microrganismos na presença de fontes de carbono. Pesq agropec bras. 24:311–319.

Soares MA, Li HY, Kowalski, KP, Bergen M, Torres MS, White, JF (2016) Functional role of bacteria from invasive *Phragmites australis* in promotion of host growth. Microb Ecol. 72:407–417.

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol. 3:1–13.

Statsoft I. (2014). Statsoft I: Statistica (data analysis software system). Version 7.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 28:2731–2739.

Tchuisseu Tchakounté GV, Berger B, Patz S, Fankem H, Ruppel S (2018) Community structure and plant growth-promoting potential of cultivable bacteria isolated from Cameroon soil. Microbiol Res. 214:47–59.

Tian B, Zhang C, Ye Y, Wen J, Wu Y, Wang H, Li H, Cai S, Cai W, Cheng Z, Lei S, Ma R, Lu C, Cao Y, Xu X, Zhang, K (2017) Beneficial traits of bacterial endophytes belonging to the core communities of the tomato root microbiome. Agric Ecosyst Environ. 247:149–156.

Vieira FCS, Nahas E (2005) Comparison of microbial numbers in soils by using various culture media and temperatures. Microbiol Res. 160:197–202.

Waghmare PR, Patil SM, Jadhav SL, Jeon BH, Govindwar SP (2018) Utilization of agricultural waste biomass by cellulolytic isolate *Enterobacter* sp. SUK-Bio. ANRES. 52:399–406.

Ying W, Yang CD, Yao Yl, Wang YQ, Zhang ZF, Li XUE (2016) The diversity and potential function of endophytic bacteria isolated from *Kobreasia capillifolia* at alpine grasslands on the Tibetan Plateau, China. J Integr Agr. 15:2153–2162.

Wollum A (1982) Cultural methods for soil microorganisms. In: Miller DRAL, Keeney RH (ed.) Methods of soil analysis. 2ed.

Wu X, Rogers Leonard B, Zhu YC, Abel CA, Head GP, Huang F (2009) Susceptibility of Cry1Ab-resistant and -susceptible sugarcane borer (*Lepidoptera*: Crambidae) to four *Bacillus thuringiensis* toxins. J Invertebr Pathol. 100:29–34.

Yoorin (2018) Fornecendo nutrientes para aumentar sua produtividade. http://www.yoorin.com.br/pt/produtos/ekosil.

Zamil SS, Ahmad S, Choi MH, Yoon SC (2010) Production of poly-N-acetylglucosamine by *Staphylococcus saprophyticus* BMSZ711: Characterization and production optimization. Bioresour Technol. 101:7177–7180.

Zhang C, Kong F (2014) Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. Appl Soil Ecol. 82:18–25.

Zhou D, Huang XF, Chaparro JM, Badri DV, Manter DK, Vivanco JM, Guo J (2016) Root and bacterial secretions regulate the interaction between plants and PGPR leading to distinct plant growth promotion effects. Plant Soil. 401:259–272.

CAPÍTULO 3 - Sugarcane as a reservoir of maize growth-promoting rhizobacteria[2]

Abstract - Plant growth-promoting rhizobacteria have several abilities to promote plant growth. Some studies show that strains specific to a plant species may have a close interface with a different plant species. In this sense, the present study aimed to analyze the bacterial strains of the sugarcane rhizosphere in promoting maize and sugarcane crops' growth. The experiments were carried out in pots, with maize and sugarcane planting in randomized blocks with eight treatments and three repetitions. Afterward, biometric and dry mass analyses were carried out on the plants, counting the number of bacteria on the plants and soil and the concentration of nutrients in the soil and plants. In sugarcane, *Enterobacter* sp. IP11, *B. thuringiensis*, *Enterobacter* sp. IP14, *A. spanius*, and *B. anthracis* provided an increase in root dry matter of 14, 18, 19, 21, and 23%, respectively, to the control. *Enterobacter* sp. IP11 and *B. thuringiensis* also increased the height to the control by 11 and 14%. In maize, *Enterobacter* sp. IP14, *B. thuringiensis*, and *B. anthracis* increased the plant height by 10, 12, and 13%, respectively, to the control without inoculation. According to the results, it can be observed that most of the bacterias added from sugarcane have a preference for the same culture. However, the results also show that the sugarcane reinforcing strains can also promote a maize crop's growth effect.

Keywords: abilities, growth promotion, specificity, *Saccharum* spp., *Zea mays*.

[2] Este capítulo corresponde ao artigo científico submetido à revista Frontiers in Sustainable Food Systems e encontra-se em avaliação para publicação.

Introduction

Plant growth-promoting rhizobacteria (PGPR) include bacteria that inhabit the rhizosphere and facilitate plant growth through direct mechanisms, which include the production of phytohormones and greater availability of nutrients or indirect mechanisms such as suppression of pathogens by antibiosis, synthesis of lytic enzymes and induced systemic resistance (ISR) (Glick, 2014).

Compared to non-rhizospheric soil, rhizosphere is rich in nutrients due to the exudation and root deposits, as a result, the number of bacteria around plant roots is 10 to 100 times higher than in non-rhizospheric soil (Spaepen et al., 2009). Communication between plant roots and soil microbiota occurs through root exudates (Kloepper and Schroth, 1981), which work as communicating molecules, providing biological and physiological interactions in the rhizosphere, influencing the chemical and physical properties of soil and soil microbiota, inhibiting the growth of pathogenic species and facilitating beneficial symbiosis as in the case of PGPR selection (Nardi et al., 2000).

Studies with rhizobacterial microbial communities associated with plants and soil have shown that the specificity for each plant species can be attributed to secondary metabolites released by root exudates (Ramakrishna et al., 2019). Thus, one of the limiting factors in the use of PGPR is that when there is beneficial effect of PGPR on a given plant species, this effect may not occur in other crops due to the lower plant-microorganism interaction and consequently lower colonization and microbial rhizosphere establishment (Xu et al., 2011; Zhang et al., 2013).

There is great need to search for new bacterial isolates that promote plant growth, but for that, it is necessary to understand the mechanisms of action between plant and microorganism, since the genetic variation of the plant cultivar can affect the plant-microorganism interaction and plant development due to the interference in the mode of bacterial action (Xu et al., 2011). Consequently, bacteria isolated from a plant species may not promote plant growth when applied to other species (Reinhold et al., 1985). When growth promotion occurs by the application of PGPR in crops, this can result in better plant development and yield, contributing to sustainable agriculture by

reducing the use of mineral fertilizers and phytosanitary products (Afzal et al., 2019; Parra and Cuevas., 2002). But for this to occur, the first step is the strong interaction between plant and microorganism with microbial colonization of the rhizosphere (Zhang et al., 2013).

Despite the specificity relationships between bacterial species and plants from which these bacteria were isolated, it is possible that bacterial species isolated from a given plant species have growth-promoting effect on a different plant species, as demonstrated by Mendes et al. (2019), who used bacteria isolated from maize in sugarcane and Dias et al. (2019), who used the same isolates from maize in cotton.

In this sense, the present study aimed to analyze the bacterial strains of the sugarcane rhizosphere in promoting the growth of maize and sugarcane crops.

Materials and methods

The research was carried out at the São Paulo State University FCAV/UNESP in the municipality of Jaboticabal, SP (21º14'05"S, 48º17' 09" W and 615.01 m a.s.l.). Two experiments were carried out: one with sugarcane plants lasting 75 days and another with maize plants lasting 60 days.

Experimental design

The experimental design was the same for sugarcane and maize (Table 1). A randomized block design was used, with three replicates and eight treatments. Twenty-four pots were used, each pot being considered an experimental plot, totaling 48 pots for both cultures.

Planting

For planting sugarcane, the RB966928 variety was used. Initially, in greenhouse, minicuttings were planted (Fig. 1A) in sprouting boxes. After 15 days, already formed seedlings (Fig. 1B) were transplanted into pots and kept outdoors (Fig. 1 C) for 60 days.

Table 1. Treatments used in experiments with sugarcane and maize and accession number of rhizobacteria's sequences deposited at NCBI.

Treatments	Inoculants	Accession number
T1	*Staphylococcus saprophyticus* IJ8	MT764797.1
T2	*Enterobacter* sp. IP11	MT764798.1
T3	*Enterobacter* sp. IP14	MT764799.1
T4	*Bacillus anthracis* IP17	MT764800.1
T5	*Bacillus thuringiensis* IP21	MT764801.1
T6	*Achromobacter spanius* IP23	MT764802.1
T7	*Enterobacter asburiae* IP24	MT764803.1
T8	Controle (Sem inoculação)	-

Minicuttings were planted in eight sprouting boxes, one intended for treatment. Sprouting boxes were filled with 1:1 sand and vermiculite. The germination of minicuttings was carried out in order to ensure that sugarcane seedlings had the same vegetative stage of development at the time of planting in pots.

For the planting of maize (*Zea mays* L., hybrid 2B587PW Dow Agro sciences), four seeds were sown and after germination, thinning was performed, leaving only one plant per pot.

Sugarcane seedlings (Fig. 1C) and maize seeds (Fig. 1D) were added to pots with volumetric capacity of 5 liters and filled with 2 cm of crushed stone for water drainage and sieved soil classified as Eutrophic Red Latosol. The average maximum and minimum temperatures during the period were 27.3 and 13.5 ºC, respectively.

The maize irrigation was performed manually, with a water volume of 700 mm during the period of conduction of the experiment. And for sugarcane, the water volume was 620 and 196 mm, supplied via irrigation and precipitation, respectively.

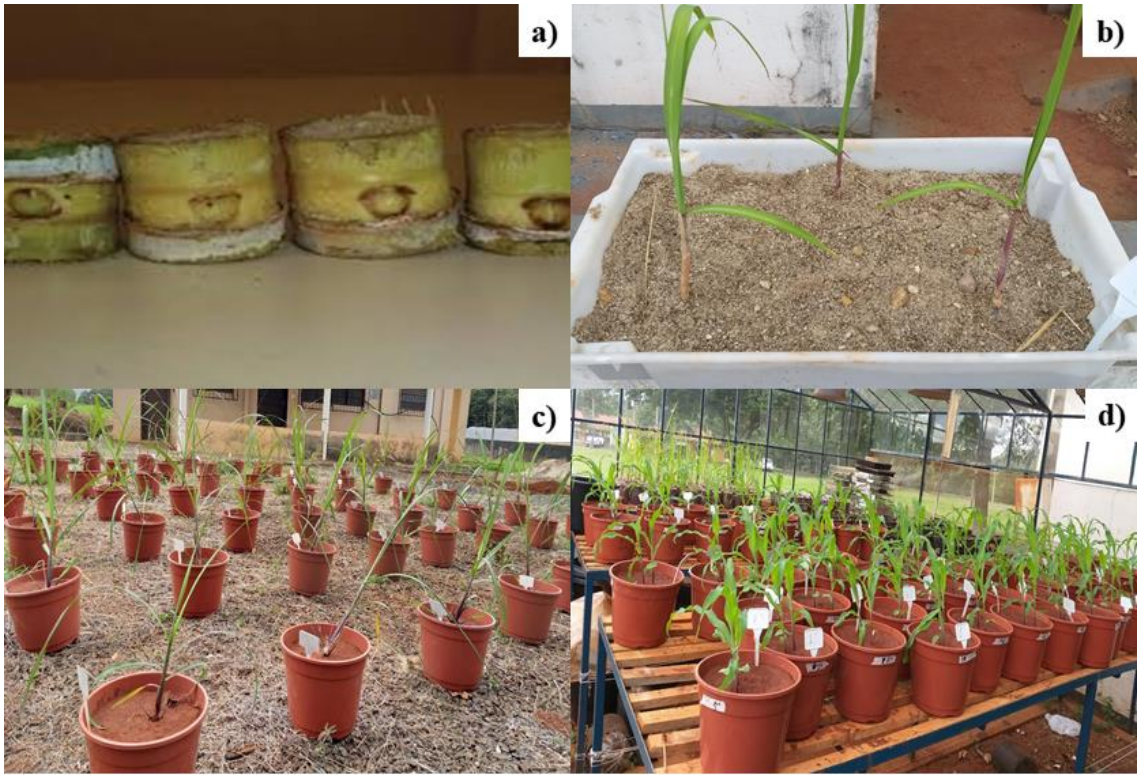

Figure 1. a) minicuttings; b) sprouting boxes with 15-day-old sugarcane; c) sugarcane plants placed outdoors; d) maize plants in greenhouse.

Fertilizations

In sprouting boxes, no fertilization was carried out, as minicutting contains enough energy reserve for the germination of buds.

N, P, K, Mg, Bo and Zn were used as sources of urea, simple superphosphate, potassium chloride, manganese sulfate, boric acid and zinc sulphate, respectively. Fertilization was carried out individually for each pot, and homogenization was performed with the aid of plastic bag. Chemical analysis and doses used for both cultures are described in Table 2.

Bacterial inoculation

Bacterial strains used in the present study were isolated from sugarcane rhizosphere, identified by automatic sequencing of the 16S ribosomal gene (Santos; Rigobelo, 2020) and stored lyophilized in Brain Heart Infusion medium in freezer at -20 ºC. Strains belong to the collection of microorganisms from the Laboratory of Soil Microbiology (LSM) of FCAV/UNESP, Jaboticabal and their sequences are available in the NCBI database (Table 1).

Table 2. Chemical analysis of soil before planting used for filling pots and doses of mineral fertilizers used for sugarcane and maize in pots. Doses were calculated based on the analysis of soil and culture.

Soil chemical analysis				
O.M. = 10 g/ dm^3; P resin = 14 mg/ dm^3; K = 0.7 mmolc/ dm^3; Ca = 79 mmolc/ dm^{-3}; Mg = 13 mmolc/dm^3; SB = 93.4 mmolc/dm^3; pH in $CaCl_2$ = 6.9; cation exchange capacity (CEC) of 104.2 mmolc/dm^{-3}; V of 90%[2]				
Culture	**Sugarcane**		**Maize**	
Fertilizer	Recommended (Kg ha^{-1})	Applied per pot (g)	Recommended (Kg ha^{-1})	Applied per pot (g)
Urea	30	0.37	30	0.37
Simple Superphosphate	140	4.16	100	3.08
Potassium chloride	200	1.89	50	0.47
Zinc sulphate	5	0.15	4	0.47
Boric acid	2	0.065	-	-
Manganese sulphate	3	0.057	-	-

Inoculants were obtained after resuspension of each lyophilized bacterial strain for 24h incubation in BOD oven at 28 ºC using nutrient broth until final concentration of 1 x 10^9 colony-forming units (CFU) mL^{-1}.

The first inoculation in sugarcane was carried out after planting seedlings in pots and in maize seven days after planting. Subsequently, both crops received inoculation via soil with the aid of graduated pipette, with the addition of 15 mL of inoculum per pot, every 15 days totaling four inoculations. In the control treatment, no inoculum was added.

Assessments

Biometric analysis and bacterial count

In sugarcane, the height of the main tiller was measured, performed from the base of the plant up to leaf +1 (Kuijper's numbering system). In maize plants, height was verified by measuring from the base of the plant to the apex of the flag leaf.

For the counting of endophytic bacteria, plants were separated into shoots and roots, which were washed with the aid of water jet to remove soil. Subsequently, 1 g of each vegetable tissue collected (shoots and roots) was weighed and submitted to a superficial disinfection procedure in order to eliminate epiphytic microorganisms. In this process, both parts were sequentially immersed in 70% ethanol for 1 minute, 3% sodium hypochlorite solution for 3 minutes and 70% ethanol for 30 seconds. Subsequently, three rinses were performed in sterile distilled water. Finally, shoots and roots were aseptically macerated with the aid of mortar and pistil, which were placed in Erlenmeyer flask containing 9 mL of 0.1% NaCl.

For the counting of bacteria present in soil, 10 g of rhizospheric soil were added in Erlenmeyer containing 95 mL of 0.1% sodium pyrophosphate saline solution. All Erlenmeyer flasks were stirred for 1 hour and then serial dilutions were performed, inoculating 100 µL of triplicate diluted into Petri dish containing agar nutrient medium. Plates were kept in BOD oven at 30 ºC, counting the amount of CFU after 24, 48 and 72 hours (Vieira; Nahas 2005).

Vegetable dry matter and soil and plant nutrients

Dry root matter (RDM), shoot dry matter (SDM), and total dry matter (TDM), was measured separating plants into shoots and roots, which were washed with the aid of water jet to remove soil. Both parts were added in paper bags and taken to oven at

65ºC until they reached constant mass. After drying, mass was measured on semi-analytical scale. To obtain TDM, RDM and SDM were added.

The phosphorus content in resin in soil was determined using spectrophotometry (Malavolta, 1989), total nitrogen according to Tedesco et al. (1995) with sulfuric digestion (H_2SO_4), followed by distillation and titration.

Shoot and root samples used for dry matter determination were ground and then used to determine phosphorus and nitrogen levels. Phosphorus contents were determined by nitric-perchloric digestion followed by spectrophotometric analysis (Marino et al., 1995), and nitrogen contents by sulfuric digestion followed by titration (Malavolta et al., 1989).

Data analysis

Data were submitted to analysis of variance (F Test) comparing the averages of treatments by the Duncan test at 5% probability, using the Agroestat version 1.0 software (Barbosa; Maldonato, 2010).

Results

Sugarcane

B. thuringiensis IP21 and *Enterobacter* sp. IP11 isolates promoted the greatest height of sugarcane plants, providing increase of 14.1 and 10.4%, respectively, in relation to control (Fig. 2A).

For SDM, RDM and TDM, *Enterobacter* sp. IP14, *B. anthracis* IP17, *A. spanius* IP23 were superior compared to control. Furthermore, *B. thuringiensis* IP21 was

superior for RDM and TDM, and *Enterobacter* sp. IP11 isolate also increased TDM (Fig. 2C, 2E and 2G).

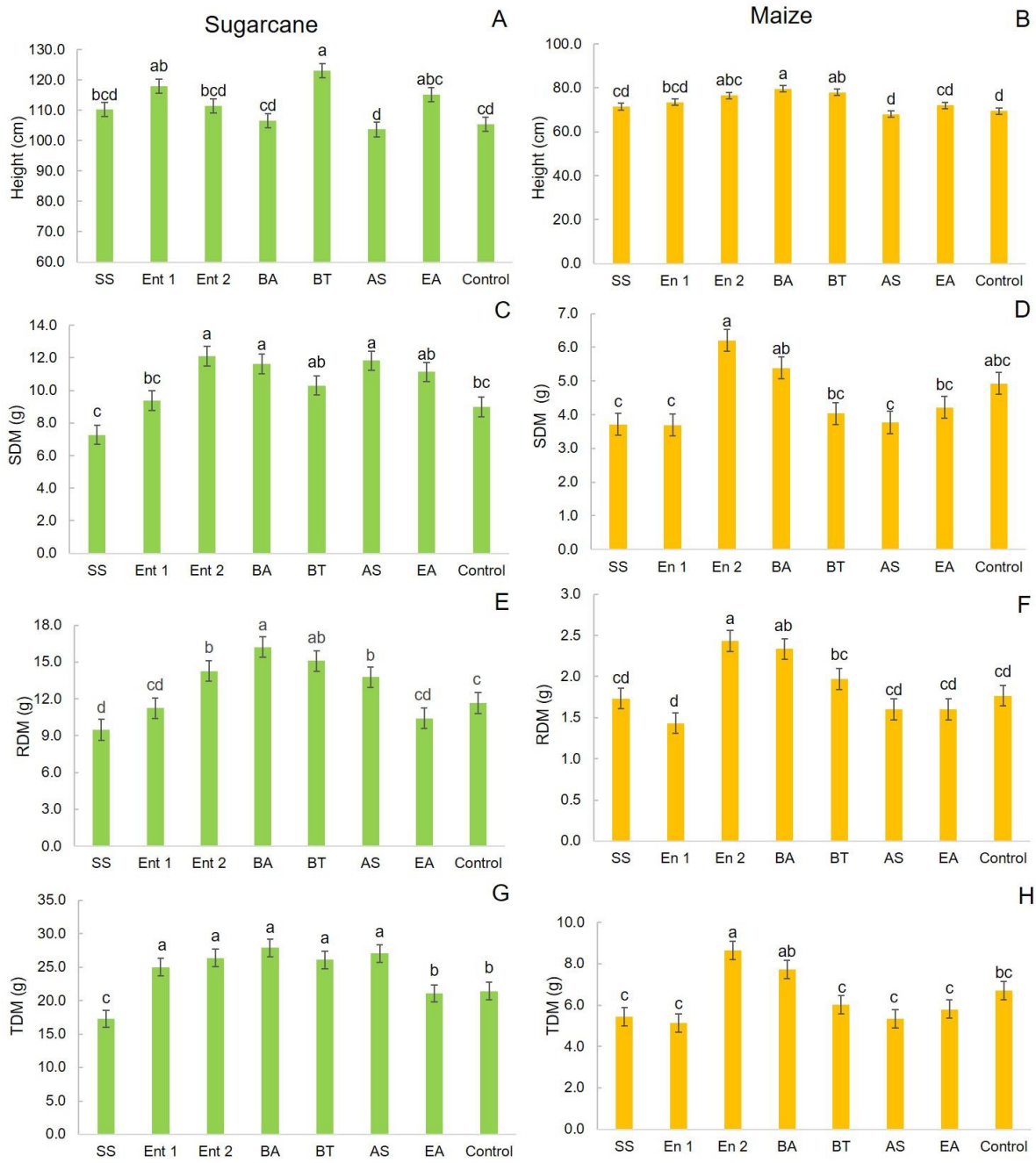

Figure 2. Biometric data for: (A) in sugarcane (B) and maize height (C) in sugarcane (D) and maize SDM (E) in sugarcane (F) and maize RDM (G) in sugarcane (H) and maize TDM. Averages followed by equal letters do not differ by the Duncan's test at 5% probability.

*SS: *S. saprophyticus* IJ8; Ent 1: *Enterobacter* sp. IP11; Ent 2: *Enterobacter* sp. IP14; BA: *B. anthracis* IP17; BT: *B. thuringiensis* IP21; AS: *A. spanius* IP23; EA: *E. asburiae* IP24; Control: No inoculation.

As for the amount of CFU in shoots, control treatment was statistically lower than treatments with *S. saprophyticus* IJ8, *Enterobacter* sp. IP11, *Enterobacter* sp. IP14, *B. anthracis* IP17 and *A. spanius* IP23 (Fig. 3A). In roots, the amount of CFU was higher in treatment with *Enterobacter* sp. IP14 and *E. asburiae* IP24, differing from control (Fig. 3C). For the amount of CFU in soil, *B. anthracis* IP17 showed higher amount, being statistically greater than control (Fig. 3E).

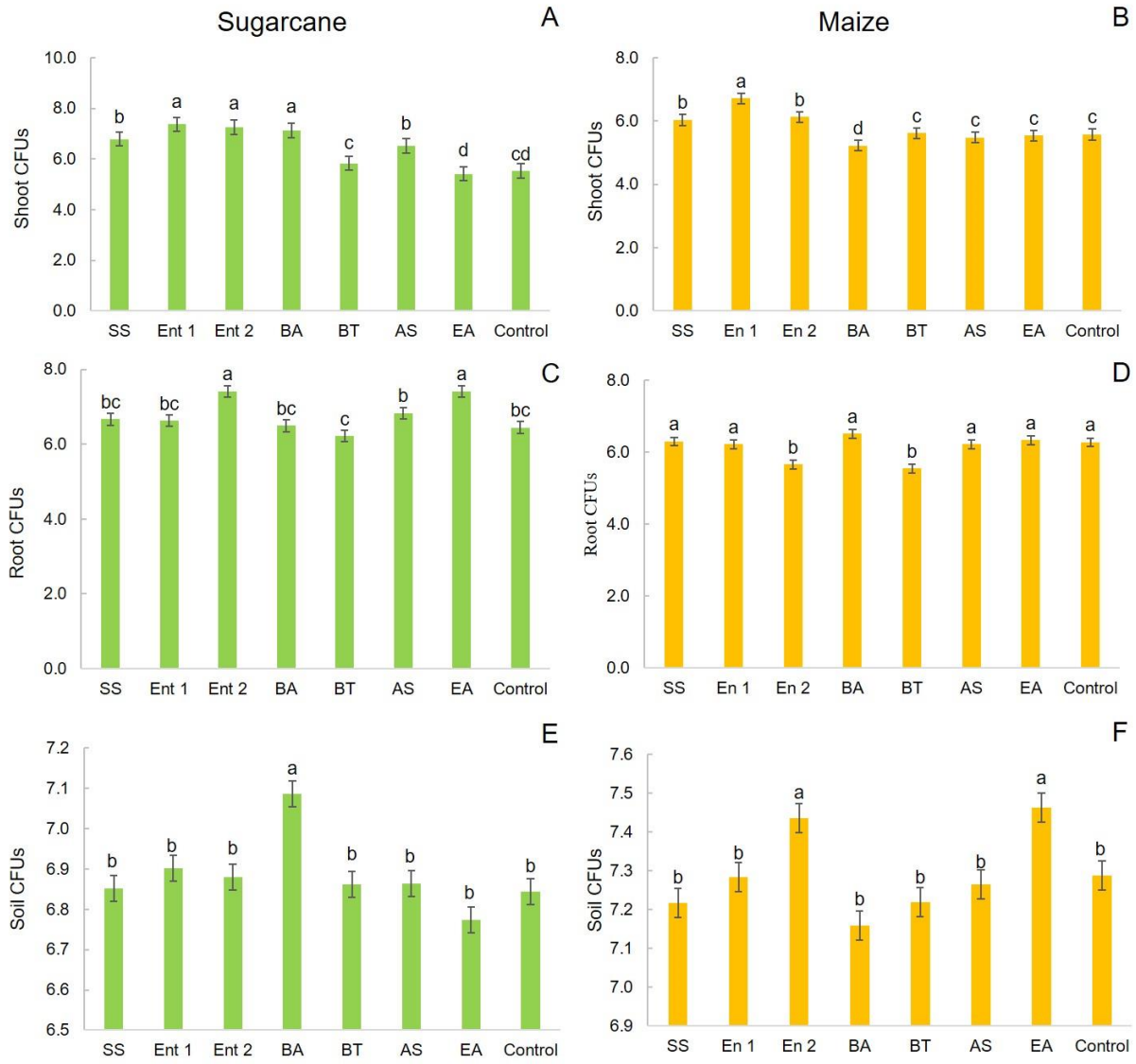

Figure 3. Number of CFU: (A) in sugarcane (B) and maize shoots (C) in sugarcane (D) and maize roots (E) in soil with sugarcane (F) and maize. Means followed by equal letters do not differ by the Duncan test at 5% probability.
*SS: *S. saprophyticus* IJ8; Ent 1: *Enterobacter* sp. IP11; Ent 2: *Enterobacter* sp. IP14; BA: *B. anthracis* IP17; BT: *B. thuringiensis* IP21; AS: *A. spanius* IP23; EA: *E. asburiae* IP24; Control: No inoculation. **Data transformed into log 10.

The amount of N and P extracted by sugarcane shoots was higher in treatments that received *Enterobacter* sp. IP14, *B. anthracis* IP17, *A. spanius* IP23 and *E. asburiae* IP24 inoculations, thus being statistically greater than control without inoculation (Fig 4A and 5A).

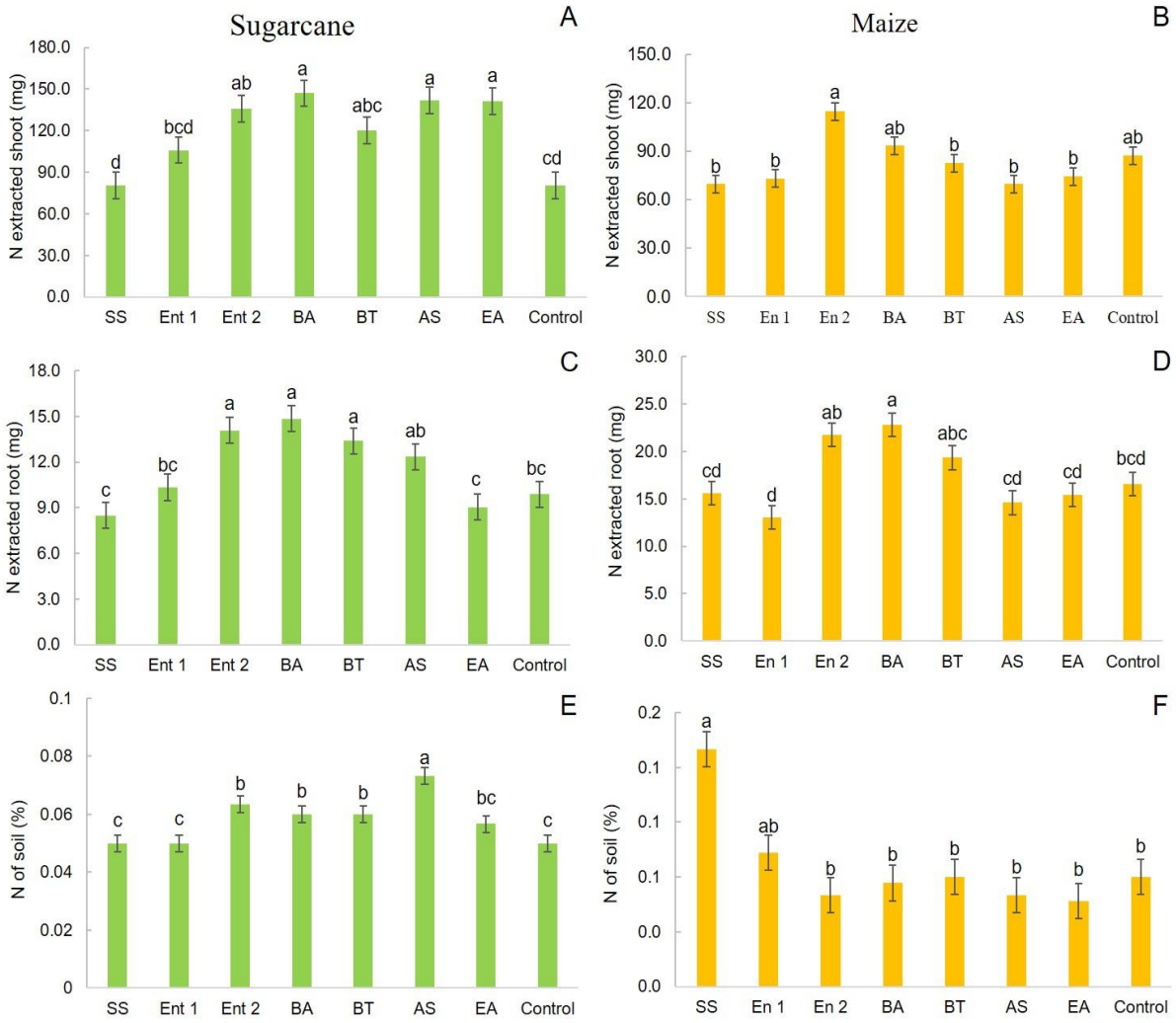

Figure 4. N concentration: (A) extracted by sugarcane (B) and maize shoots; (C) extracted by sugarcane (D) and maize roots (E) in soil with sugarcane (F) and maize. Means followed by equal letters do not differ by the Duncan's test at 5% probability.

*SS: *S. saprophyticus* IJ8; En 1: *Enterobacter* sp. IP11; En 2: *Enterobacter* sp. IP14; BA: *B. anthracis* IP17; BT: *B. thuringiensis* IP21; AS: *A. spanius* IP23; EA: *E. asburiae* IP24; Controle: Sem inoculação.

Enterobacter sp. IP14, *B. anthracis* IP17 and *B. thuringiensis* IP 21 favor the extraction of N and P in roots, and *A. spanius* IP23 is also responsible for greater

extraction of P in the root system (4C and 5C). As for the N concentration in soil, the result was equivalent to P extraction by roots, in such a way that the same strains stood out (4E). Regarding P in soil, only *E. asburiae* IP24 increased P concentration, differing from control (5E).

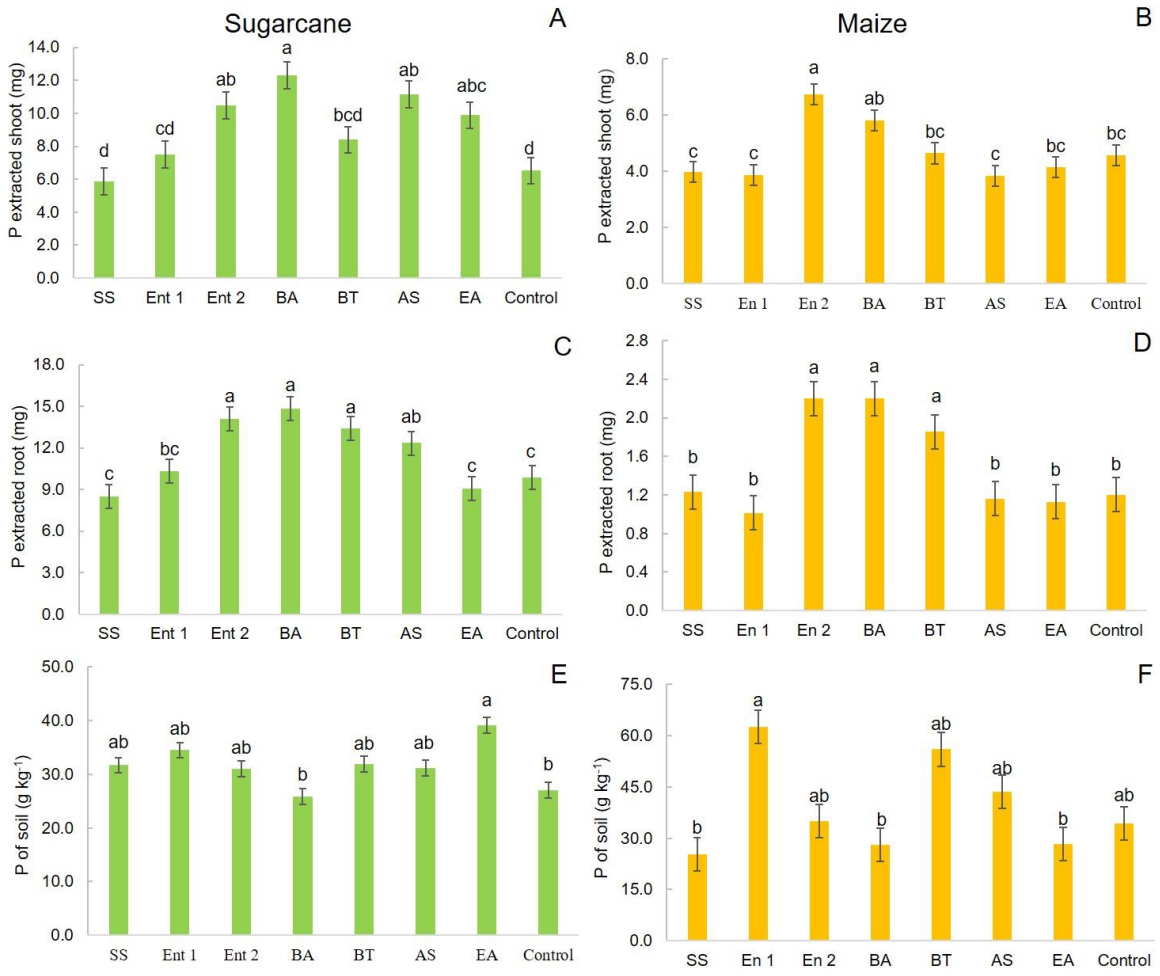

Figure 5. P concentration: (A) extracted by sugarcane (B) and maize shoots (C) extracted by sugarcane (D) and maize roots (E) in soil with sugarcane (F) and maize. Means followed by equal letters do not differ by the Duncan's test at 5% probability.

*SS: *S. saprophyticus* IJ8; En 1: *Enterobacter* sp. IP11; En 2: *Enterobacter* sp. IP14; BA: *B. anthracis* IP17; BT: *B. thuringiensis* IP21; AS: *A. spanius* IP23; EA: *E. asburiae* IP24; Controle: Sem inoculação.

Maize

Plant height was influenced by *B. anthracis* IP17, *B. thuringiensis* IP21 and *Enterobacter* sp. IP14 inoculations with increments of 12.6%, 10.9% and 9.2% in relation to control, respectively in plant height (Fig. 2B).

For SDM, no significant differences were observed in relation to rhizobacteria inoculations and control treatment. However, *Enterobacter* sp. IP14 inoculation was superior to *S. saprophyticus* IJ8, *Enterobacter* sp. IP11, *B. thuringiensis* IP21, *A. spanius* IP23, *E. asburiae* IP24 inoculations (Fig. 2D).

Enterobacter sp. IP14 and *B. anthracis* IP17 provided increase of 27.5% and 24.5%, respectively, for RDM, in relation to control treatment. For TDM, *Enterobacter* sp. IP14 stood out, increasing 22.6%, being superior to control, without inoculation (Fig. 2F e 2H).

For CFU in shoots, the highest concentration was found in plants inoculated with *Enterobacter* sp. IP11, followed by *S. saprophyticus* IJ8 and *Enterobacter* sp. IP14, being superior to control (Fig. 3B).

Enterobacter sp. IP14 and *B. thuringiensis* IP 21 were statistically inferior to all other treatments used, regarding the amount of CFU in maize roots (Fig. 3D), while for CFU in soil, *E. asburiae* IP24 and *Enterobacter* sp. IP14 were found in greater amounts (Fig. 3F).

In shoots, there was no statistical difference for extracted N concentration (Fig. 4B). In roots, treatment inoculated with *B. anthracis* IP17 increased the extracted N concentration by 27.4% compared to control (Fig. 4D). In relation to N in soil, *S. saprophyticus* IJ8 increased the N concentration by 53.8% in relation to control (Fig. 4F).

Enterobacter sp. IP14 increased the P concentration extracted by maize shoots by 32.2%, in relation to control (Fig. 5B). For P extracted by roots, the increase was 45.5% in plants inoculated with *B. anthracis* IP17 and *Enterobacter* sp. IP14 and 35.5% for *B. thuringiensis* IP21, compared to control (Fig. 5D). Regarding P in soil, there was no significant difference between treatments and control (Fig. 5F).

Discussion

Bacteria used in the present study were isolated from sugarcane culture and showed different behavior between cultures and variables observed. In maize, treatment that received *Enterobacter* sp. IP14 showed the highest total number of bacteria in shoots and in soil compared to control, not differing from roots (Fig. 3B and 3D), showing the site preference for colonization of this bacterium. Consequently, maybe due to the colonization of these environments (shoots and soil), *Enterobacter* sp. IP14 promoted increases in plant development such as height, RDM and TDM. On the other hand, in maize, *B. anthracis* IP17 also promoted increase in height and RDM (Fig. 2B and Fig. 2F) and, surprisingly, did not increase colonization of shoots (Fig. 3B), and also did not increase colonization in soil compared to control.

At first analysis, these results might seem contradictory, but strongly suggest that the colonization of soil, roots and shoots by plant growth-promoting bacteria is related to the presence of these bacteria in these environments and not necessarily to a greater number of total bacteria in relation to control. Each treatment received four inoculations and at the end of the experiment, the total number of bacteria in soil, roots and shoots were measured and higher number of bacteria was expected in treatments that received inoculations compared to control. Similarly, to *B. anthracis*, the total number of bacteria in different environments did not increase in relation to control; however, bacteria promoted plant growth. The first step in promoting plant growth is the rhizosphere colonization and then several factors related to the plant-bacterium interaction and the colonization of plant tissues such as roots and leaves (Bulgarelli et al., 2013).

Bentes et al. (2019) evaluated *B. subtilis* in maize and found that the only isolate that promoted an increase in yield was also the only one that did not differ in total number of bacteria (CFU) compared to control. This result strongly suggests that the bacterial establishment of rhizosphere, roots and leaves does not require a large number of bacteria, but rather a small presence added to high efficiency and close isolate-plant interaction.

For sugarcane, there were more treatments that showed that the bacterial strains used can be considered growth promoters, namely, *Enterobacte*r sp. IP11, *Enterobacter* sp. IP14, *B. anthracis* IP17, *B. thuringiensis* IP21 and *A. spanius* IP23. All these strains were able to successfully colonize shoots, roots or soil, except for *B. thuringiensis* IP21. It is interesting to highlight that the strains used were rhizobacteria isolated from sugarcane, demonstrating a certain specificity of these bacteria to the culture from which they were isolated.

Plants can affect microbial populations in the soil, where each plant species selects specific microbial populations and root exudates are a driving force in this process (Bais et al., 2006; Haichar et al., 2008). The composition of root exudates varies from plant to plant and affects the relative abundance of microorganisms around roots (Somers et al., 2004).

Studies have demonstrated that the specificity of root colonization occurs through the adsorption (or anchoring) of bacteria on root surface. In addition, competition ability varies according to exudates synthesized by plants, in which bacterial colonization occurs in the depression between epidermal cells (Pinheiro et al., 2002). Once in roots, bacteria cause changes in root hair density and length, resulting in an increase in the surface of the root system and allowing better exploitation of soil nutrients and water (Dobbelaere et al., 2003). Such effects are associated with the ability to release substances that promote growth such as indolacetic acid (IAA), gibberellins and cytokinins (Berg; Smalla, 2009).

Competitive rhizosphere colonization and establishment in the root zone are important for the success of rhizosphere organisms (Weller, 1988; Lugtenberg et al., 2002; reviewed in Compant et al., 2005). Colonization steps include recognition, adhesion, invasion (endophytes and pathogens only), colonization and growth including various strategies for establishing interaction. Plant roots initiate cross-conversations with soil microorganisms, producing signals recognized by microorganisms, which in turn, produce signals that initiate colonization (Bais et al., 2006).

Plant genotype influences the composition and size of microbial communities associated with plants through specific metabolic processes (Garbeva et al., 2008; Philippot et al., 2013; Bouffaud et al., 2011). However, the combined activities of

microbial communities in the rhizosphere are more important for plant development than the role of specific groups in the rhizosphere. In a way, when a bacterial isolate promotes plant growth, it is not actually its effect alone, but the result of the interaction of this bacterial isolate with the microbial communities of the rhizosphere.

The host specificity seems to be controlled both by the specific bacterial adaptation of the strain and by the non-specific characteristics of the host plant. Thus, the ability to colonize and stimulate the growth of a restricted group of plants, by bacteria, may involve mechanisms involved in host recognition and root colonization, in addition to strict control of beneficial bacterial properties by plant compounds (Bergand Smalla, 2009).

Discrepancies in chemotaxis have been reported for *Azotobacter* spp., which when submitted to the stress test, two strains were preferentially attracted by wheat exudates and seven others by cotton exudates, and the observed differences were attributed to specific plant variations in the energy yield of exudated organic acids (Kumar et al., 2007).

In a review study, it was shown that the genus *Azospirillum* was able to promote the growth of 113 plant species in 35 botanical families, including 14 cereal species, concluding that the genus *Azospirillum* is not specific for cereals and that it could be used as growth promoter for all plant species tested so far. Given the scarcity of widespread screening, the affinity of strains to a genotype, cultivar or plant species cannot be nullified (Pereg et al., 2016).

Previous studies have determined the specific chemotactic responses of bacterial strains to specific organic acids, amino acids and aromatic compounds, which are excreted by different plant species. *Azospirillum brasilense* SpT60 (isolate from wheat roots) was strongly attracted by D-fructose, L-aspartate, oxalate and citrate, whereas *A. brasilense* JM6A2 (isolate from maize roots) did not respond or responded weakly to these compounds. Instead, it was attracted by L-malate (Finkemeier and Sweetlove, 2009; Reinhold et al., 1985). Therefore, a plant that excreted D-fructose, L-aspartate, oxalate and citrate, would be able to attract *A. brasilense* SpT60 to its rhizosphere, facilitating its colonization, and would not attract *A. brasilense* JM6A2.

Bacterial and host genotypes have been described as influencing root colonization, which process is probably mediated by genetic determinants of both.

These differences in root colonization can occur due by the presence of genes involved in this process or by a change in the expression of genes present in all bacterial strains / plant genotypes. In both mechanisms, the formation of a physical interaction between bacteria and plant suggests specificity between components of the bacterial surface and the plant's receptors on the root surface (Drogue et al., 2012).

Species *B. anthracis*, *B. thuringiensis*, *A. spanius* and *E. asburiae* used in the present study did not promote the growth of maize plants, differently from sugarcane, suggesting that the lack of plant growth by these bacterial isolates was due to the lack of interaction with the host and not to inability of isolates to promote plant growth (Frank et al., 2017).

In the present study, two different *Enterobacter* sp. strains were used, one of which, *Enterobacter* sp. IP14, promoted growth in both maize and sugarcane, and the other, *Enterobacter* sp. IP11, promoted growth only in sugarcane. In the case of maize, growth promotion is reflected in the increase of fresh and dry plant biomass, which is associated with efficient nitrogen fixation, phosphorus solubilization, production of IAA and siderophores by bacteria (Youseif, 2018).

In review Milani et al. (2019) used several *B. subtilis* strains in maize culture and despite being the same bacterial species, it was observed that some strains promoted plant growth while others did not, demonstrating that there may be differences regarding growth promotion within the same species and that this growth-promoting effect is isolate dependent.

The smaller number of isolates efficient in the growth of maize plants demonstrates that plant genotype is of great importance in promoting growth (Haichar et al., 2008; Berg and Smalla, 2009), and that, the strength of the rhizosphere effect may differ between plant species and that the plant species from which the bacteria were isolated may promote a closer relationship with isolates compared to other species (Bulgarelli et al., 2013).

According to results, it could be concluded that most bacteria isolated from sugarcane had preference for the same culture, even though being a different genotype. However, it is possible and was observed in this study that a small number of bacteria isolated from sugarcane promote maize growth and development, suggesting that sugarcane is a reservoir of isolates.

References

Afzal, I., Shinwari, Z. K., Sikandar, S., Shahzad, S. (2019). Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Microbiol. Res. 221, 36-49. doi:10.1016/j.micres.2019.02.001

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., Vivanco, J. M. (2006). "The role of root exudates in rhizosphere interations with plants and other organisms", in Annual Review of Plant Biology, (Palo Alto), 233-266. doi:10.1146/annurev.arplant.57.032905.105159

Barbosa, J., C, Maldonado, J .W. (2010). AgroEstat: sistema para análises estatísticas de ensaios agronômicos. Jaboticabal: Departamento de Ciências Exatas.

Berg, G., Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68,1-13. doi:10.1111/j.1574-6941.2009.00654.x

Bouffaud, M. L., Kyselkova, M., Gouesnard, B., Grundmann, G., Muller, D., Moenne-Loccoz, Y. (2012). Is diversification history of maize influencing selection of soil bacteria by roots? Mol. Ecol. 21,195-206. doi:10.1111/j.1365-294X.2011.05359.x

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E. V. L., Schulze-Lefert, P. (2013). "Structure and functions of the bacterial microbiota of plants", in: Annual Review of Plant Biology, ed. S. S. Merchant (Palo Alto), 807-838. doi:10.1146/annurev-arplant-050312-120106

Compant, S., Duffy, B., Nowak, J., Clement, C., Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 71, 4951-4959. doi:10.1128/aem.71.9.4951-4959.2005

Diaz, P. A. E., Baron, N. C., Rigobelo, E. C. (2019). *Bacillus* spp. as plant growth-promoting bacteria in cotton under greenhouse conditions. Aust. J. Crop Sci. 13, 2003-2014. doi: 10.21475/ajcs.19.13.12.p2003

Dobbelaere, S., Vanderleyden, J., Okon, Y. (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit. Rev. Plant. Sci. 22, 107-149. doi:10.1080/713610853

Drogue, B., Dore, H., Borland, S., Wisniewski-Dye, F., Prigent-Combaret, C. (2012). Which specificity in cooperation between phytostimulating rhizobacteria and plants? Res. Microbiol. 163, 500-510. doi:10.1016/j.resmic.2012.08.006

Finkemeier, I., Sweetlove, L. J. (2009). The role of malate in plant homeostasis, 1 -3.

Frank, A. C., Guzman, J. P. S., Shay, J. E. (2017). Transmission of bacterial endophytes. Microorganisms. 5, 1: 21. doi:10.3390/microorganisms5040070

Garbeva, P., van Elsas, J. D., van Veen, J. A. (2008). Rhizosphere microbial community and its response to plant species and soil history. Plant and Soil. 302, 19-32. doi:10.1007/s11104-007-9432-0

Glick, B. R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol. Res. 169, 30-39. doi:10.1016/j.micres.2013.09.009

Haichar, F. E. et al. (2008). Plant host habitat and root exudates shape soil bacterial community structure. ISME J. 2, 1221-1230. doi:10.1038/ismej.2008.80

Kloepper, J. W., Schroth, M. N. (1978). Plant growth-promoting rhizobacteria on radishes. Proc of the 4th Internet Conf on Plant Pathogenic Bacter, Station de Pathologie Vegetale et Phytobacteriologie, INRA, Angers, France, 2, 879- 882.

Kumar, R., Bhatia, R., Kukreja, K., Behl, R, K., Dudeja, S. S., Narula, N. (2007). Establishment of *Azotobacter* on plant roots: chemotactic response, development and analysis of root exudates of cotton (*Gossypium hirsutum* L.) and wheat (*Triticum aestivum* L.) J. Basic. Microbiol. 47, 436-439. doi:10.1002/jobm.200610285

Malavolta, E., Vitti, G. C., Oliveira, S. A. d. (1997). Avaliação do estado nutricional das plantas. Princípios e aplicações.

Milani, R., Santos, R. M., Bentes, L. L., Kandasamy, S., Lazarovits, G., Rigobelo, E. C. (2019). *Bacillus subtilis* isolates with different abilities to promote plant growth in maize, cotton and soybean crops isolation and characterization of bacterial strains. Asian Jr. of Microbiol. Biotech. Env. Sc. 21, 827-836.

Nardi, S., Concheri, G., Pizzeghello, D., Sturaro, A., Rella, R., Parvoli, G. (2000). Soil organic matter mobilization by root exudates. Chemosphere. 4, 653-658. doi:10.1016/s0045-6535(99)00488-9

Parras, Y. C. F. (2002). Revisión bibliográfica Potencialidades de *Azospirillum* como inoculante para la agricultura. Cult. trop. 23, 31-41.

Pereg, L., de-Bashan, L. E., Bashan, Y. (2016). Assessment of affinity and specificity of *Azospirillum* for plants. Plant Soil. 399, 389-414. doi:10.1007/s11104-015-2778-9

Philippot, L., Raaijmakers, J. M., Lemanceau, P., van der Putten, W. H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789-799. doi:10.1038/nrmicro3109

Pinheiro, R. D., Boddey, L. H., James, E. K., Sprent, J. I., Boddey, R. M. (2002). Adsorption and anchoring of *Azospirillum* strains to roots of wheat seedlings. Plant Soil. 246, 151-166. doi: 10.1023/A:1020645203084

Raij, B. V., Cantarella, H., Quaggio, J. A., Furlani, Â. M. C. (1997). Boletim técnico n.° 100. Recomendações de adubação e calagem para o estado de São Paulo. 2 ed. Instituto Agronômico, Campinas, São Paulo, Brasil.

Ramakrishna, W., Yadav, R., Li, K. F. (2019). Plant growth promoting bacteria in agriculture: Two sides of a coin. Appl. Soil. Ecol. 138, 10-18. doi:10.1016/j.apsoil.2019.02.019

Reinhold, B., Hurek, T., Fendrik, I. (1985). Strain-Specific Chemotaxis of *Azospirillum* spp. J. Bacteriol. 162, 190-195. doi:10.1128/jb.162.1.190-195.1985

Santos, R. M., Kandasamy, S., Rigobelo, E. C. (2018). Sugarcane growth and nutrition levels are differentially affected by the application of PGPR and cane waste MicrobiologyOpen. 7, 1-9. doi:org/10.1002/mbo3.617

Santos, R. M., Rigobelo, E. C. (2020). Selection of Saccharum spp. rhizobacteria with growth-promoting properties using PCA analysis. Aust. J. Crop Sci., 14, 1186-1194. doi: 10.21475/ajcs.20.14.07.p2698

Somers, E., Vanderleyden, J., Srinivasan, M. (2004). Rhizosphere bacterial signalling: A love parade beneath our feet. Crit. Rev. Microbiol. 30, 205-240. doi:10.1080/10408410490468786

Spaepen, S., Vanderleyden, J., Okon, Y. (2009). “Plant growth-promoting actions of rhizobacteria” in: Advances in Botanical Research, ed. L. C. VanLoon (Academic Press Ltd-Elsevier Science Ltd, London), 283-320. doi:10.1016/s0065-2296(09)51007-5

Tedesco, M. J., Volkweiss, S. J., Bohnen, H. (1995). Análises de solo plantas e outros materiais. Porto Alegre: Departamento de solos, UFRGS.

Vieira, F. C. S., Nahas, E. (2005). Comparison of microbial numbers in soils by using various culture media and temperatures. Microbiol. Res. 160, 197-202. doi:10.1016/j.micres.2005.01.004

Weller, D. M. (1988). Biological-control of soilborne plant-pathogens in the rhizosphere with bacteria. Ann. Rev. Phytopathol. 26, 379-407. doi:10.1146/annurev.py.26.090188.002115

Wilkinson, K. G., Dixon, K. W., Sivasithamparam, K. (1989). Interaction of soil bacteria, mycorrhizal fungi and orchid seed in relation to germination of *Australian Orchids.* New Phytologist. 112, 429-435. doi:10.1111/j.1469-8137.1989.tb00334.x

Wollum, A. G. (1982). Cultural methods for soil microorganisms. Madison, Wisconsin.

Xavier, M. A. L., et al. (2014). Fatores de desuniformidade e kit de pré-brotação IAC para sistema de multiplicação de cana-de-açúcar - mudas-pré-brotadas (MPB). Campinas.

Xu, X. M., Jeger, M. J. (2013). Combined use of two biocontrol agents with different biocontrol mechanisms most likely results in less than expected efficacy in controlling foliar pathogens under fluctuating conditions: a modeling study. Phytopathology. 103, 108-116. doi:10.1094/phyto-07-12-0167-r

Youseif, S. H. (2018). Genetic diversity of plant growth promoting rhizobacteria and their effects on the growth of maize plants under greenhouse conditions. Ann. Agric. Sci. 63, 25-35 doi:10.1016/j.aoas.2018.04.002

Capitulo 4 - Considerações finais

O presente estudo abordou a importância do isolamento de novas cepas bacterianas e sua caracterização, para que essas cepas possam ser utilizadas como futuros bionoculantes. Durante a execução do trabalho foram isoladas cepas que ainda não tinham sido relatadas presentes na rizosfera da cana-de-açúcar, demonstrando o quão importante é o isolamento e caracterização de cepas.

Ainda são necessários estudos para averiguar se as cepas utilizadas na cana-de-açúcar e no milho em vaso, promoverão aumento de produtividade dessas culturas. Logo, faz-se necessário trabalhos em campo, contemplando todo o ciclo das duas culturas. No milho, as avaliações de produtividade devem incluir o número de espigas por planta, o tamanho da espiga em comprimento, o número de grãos por espiga e a massa de 1000 grãos. Na cana-de-açúcar, análises como, número de perfilhos industrializáveis, produtividade, comprimento do colmo, número de entrenós, brix, tonelada de colmo por hectare (TCH) devem realizadas. Dessa maneira pode ser concluído e comparado resultados obtivos com a execução do presente trabalho.